REMARKS

Claims 14-21 and 23-30 are pending in the application Applicants respectfully request reconsideration in view of the following remarks. Issues raised by the Examiner will be addressed below in the order they appear in the prior Office Action.

Priority. Applicants have enclosed certified copies of DE 19946301.8 and DE 19814838.0 as required by 35 U.S.C. 119(b).

Specification. The disclosure is objected to because of misspelled words and grammatical errors. Applicants have made corrections to the specification. Reconsideration and withdrawal of this objection are respectfully requested.

Claim Objections. Claims 20, 23, 25, and 26 are objected to because of informalities. Applicants have corrected the informalities as requested by the Examiner. Reconsideration and withdrawal of this objection are respectfully requested.

Rejection based on 35 U.S.C. 112, first paragraph. Claims 14-29 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. Applicants traverse this rejection to the extent it is maintained over the claims as amended.

The Office Action states that the art of treating cancer, particularly in humans, is extremely unpredictable, particularly in the case of a single compound or genus of compounds being used to treat any and all cancers. The Office Action further states that the scope of the claims is broad insofar as they claim the treatment of resistant and metastasizing tumors while providing *in vitro* biological data for only a single compound on one multidrug-resistant leukemia cell line, inhibition of metastasis for only a single compound, and the antiangiogenic effect of only a single compound. Further, the Office Action states that no specific tumors sensitive to angiogenesis inhibitors or methods of identifying such tumors are described.

Claim 14 recites a method of treating multidrug-resistant tumors or inhibiting angiogenesis or metastasis, comprising administering to a patient in need thereof, an amount of one or more N-substituted indol-3-glyoxylamides of formula I or a physiologically tolerable acid addition salt thereof effective for treating multidrug-resistant tumors or inhibiting angiogenesis or mestastasis.

With regard to the multidrug-resistant tumors, Applicants assert that even though there is biological data for only two multidrug-resistant tumor lines (L1210 and LT12MDR), multidrug-resistance is not dependent on the identity of the tumor, but rather is dependent on whether a particular drug is a substrate for a specific multidrug resistance-conferring protein or protein complex. If the drug is a substrate for such a protein or complex, then multidrug-resistance develops. Conversely, if that drug is not a substrate for the particular protein or complex, then multidrug-resistance will not develop. Accordingly, multidrug-resistance is dependent *only* on the nature of the drug, in this case an N-substituted indol-3-glyoxylamide of formula I. Multidrug-resistance is not tumor specific. Therefore, Applicants assert that by demonstrating that a compound of formula I does not act as a substrate for the protein that causes multidrug-resistance, Applicants have thereby demonstrated that compounds of formula I may treat multidrug-resistant tumors without limitation as to the particular type of tumor or the particular structure within the scope of Formula I. More specifically, Applicants would like to draw the Examiner's attention to Figure 1, which shows the development of multidrug-resistance when L1210 is exposed to any of vincristine, taxol, epothiline, or doxorubicin. Such multidrug-resistance does not inhibit the cytotoxicity of D24851, thereby indicating that D24851 is not a substrate for the protein that leads to the development of multidrug-resistance. Additionally, Applicants enclose an article dated 2001 which provides further information on the efficacy of D-24851 toward multidrug-resistant tumors (Exhibit A), and an article dated 2005 which provides further information on multidrug-resistance generally (Exhibit B). Applicants respectfully request reconsideration and withdrawal of this rejection.

With regard to the multidrug-resistant tumors, Applicants assert that even though there is biological data for only two multidrug-resistant tumor lines (L1210 and LT12MDR), multidrug-resistance is not dependent on the identity of the tumor, but rather is dependent on whether a

particular drug is a substrate for a specific multidrug resistance-conferring protein or protein complex. If the drug is a substrate for such a protein or complex, then multidrug-resistance develops. Conversely, if that drug is not a substrate for the particular protein or complex, then multidrug-resistance will not develop. Accordingly, multidrug-resistance is dependent *only* on the nature of the drug, in this case an N-substituted indol-3-glyoxylamide of formula I. Multidrug-resistance is not tumor specific. Therefore, Applicants assert that by demonstrating that a compound of formula I does not act as a substrate for the protein that causes multidrug-resistance, Applicants have thereby demonstrated that compounds of formula I may treat multidrug-resistant tumors without limitation as to the particular type of tumor or the particular structure within the scope of Formula I. More specifically, Applicants would like to draw the Examiner's attention to Figure 1, which shows the development of multidrug-resistance when L1210 is exposed to any of vincristine, taxol, epothiline, or doxorubicin. Such multidrug-resistance does not inhibit the cytotoxicity of D24851, thereby indicating that D24851 is not a substrate for the protein that leads to the development of multidrug-resistance. Additionally, Applicants enclose an article dated 2001 which provides further information on the efficacy of D-24851 toward multidrug-resistant tumors (Exhibit A), and an article dated 2005 which provides further information on multidrug-resistance generally (Exhibit B). Applicants respectfully request reconsideration and withdrawal of this rejection.

With regard to treating tumors sensitive to angiogenesis inhibitors, Applicants have amended the claims such that they now recite inhibiting angiogenesis. Further, with respect to treating metastasizing tumors, Applicants have amended the claims such that they now recite inhibiting metastasis. Finally, Applicants respectfully direct the Examiner's attention to MPEP 2164.03 which states that for a claimed genus, "representative examples together with a statement applicable to the genus as a whole will ordinarily be sufficient if one skilled in the art... would expect the claimed genus could be used in that manner without undue experimentation.... Proof of enablement will be required for other members of the claimed genus only where adequate reasons are advanced by the examiner to establish that a person skilled in the art could not use the genus as a

whole without undue experimentation." Applicants assert that the claims as amended are enabled and respectfully request reconsideration and withdrawal of this rejection.

Finally, the Office Action states that Applicant is required to amend the disclosure to include the material incorporated by reference insofar as it describes the procedures or synthetic methodology to synthesize the claimed compounds. Applicants have added the material as requested by the Examiner; therefore, Applicants respectfully request reconsideration and withdrawal of this rejection.

With regard to the abovementioned amendment to the specification, Applicants assert that the material being inserted is the material previously incorporated by reference and that the amendment to the specification contains no new matter. 37 CFR § 1.57(f). For the Examiner's convenience, Applicants have enclosed German Patent DE 196 36 150 (Exhibit C), and the corresponding US patent application serial number 08/925,326 (now U.S. patent 6,008,231), which includes a signed certification of John William Spicer that the application is a true translation into the English language of the German specification (Exhibit D).

Rejection based on 35 U.S.C. 112, first paragraph. Claims 14-29 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. Applicants traverse this rejection to the extent it is maintained over the claims as amended.

The Office Action states that claims 14, 19, and 29 recite compounds and "N-oxides" thereof and further that there is insufficient written description for said N-oxides in the specification. The Office Action further states that the compounds of the pending claims contain multiple amine functionalities and one skilled in the art is not apprised of the detailed chemical structures of compounds containing an N-oxide.

Applicants have amended the claims such that the term "N-oxide" has been removed. Applicants respectfully request reconsideration and withdrawal of this rejection.

Rejection based on 35 U.S.C. 112, second paragraph. Claims 14-29 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants traverse this rejection to the extent it is maintained over the claims as amended.

The Office Action states that claim 14 recites the administration of "an effective amount" of a compound of formula (I) which is indefinite because it is not clear what the amount being administered is effective for. Applicants have amended claim 14 such that the term "an effective amount of" has been replaced with "an amount of one or more N-substituted indol-3-glyoxylamides of formula I or a physiologically tolerable acid addition salt thereof effective for treating multidrug-resistant tumors or inhibiting angiogenesis or metastasis." Applicants respectfully request reconsideration and withdrawal of this rejection.

In view of the foregoing amendments and remarks, Applicants submit that the pending claims are in condition for allowance. Early and favorable reconsideration is respectfully solicited. Applicant believes no fee is due with this response, aside from the fees due in connection with the Petition for Extension of Time. However, if a fee is due, please charge our Deposit Account No. 18-1945, under Order No. ZIPH-009-102 from which the undersigned is authorized to draw.

Dated: April 4, 2007

Respectfully submitted,

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Exhibit A

[CANCER RESEARCH 61, 392–399, January 1, 2001]

D-24851, a Novel Synthetic Microtubule Inhibitor, Exerts Curative Antitumoral Activity *in Vivo*, Shows Efficacy toward Multidrug-resistant Tumor Cells, and Lacks Neurotoxicity[1]

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ABSTRACT

N-(pyridin-4-yl)-[1-(4-chlorbenzyl)-indol-3-yl]-glyoxyl-amid (D-24851) is a novel synthetic compound that was identified in a cell-based screening assay to discover cytotoxic drugs. D-24851 destabilizes microtubules and blocks cell cycle transition specifically at G_2-M phase. The binding site of D-24851 does not overlap with the tubulin binding sites of known microtubule-destabilizing agents like vincristine or colchicine. *In vitro*, D-24851 has potent cytotoxic activity toward a panel of established human tumor cell lines including SKOV3 ovarian cancer, U87 glioblastoma, and ASPC-1 pancreatic cancer cells. *In vivo*, oral D-24851 treatment induced complete tumor regressions (cures) in rats bearing Yoshida AH13 sarcomas. Of importance is that the administration of curative doses of D-24851 to the animals revealed no systemic toxicity in terms of body weight loss and neurotoxicity in contrast to the administration of paclitaxel or vincristine. Interestingly, multidrug-resistant cell lines generated by vincristine-driven selection or transfection with the M_r 170,000 P-glycoprotein encoding cDNA were rendered resistant toward paclitaxel, vincristine, or doxorubicin but not towards D-24851 when compared with the parental cells. Because of its synthetic nature, its oral applicability, its potent *in vitro* and *in vivo* antitumoral activity, its efficacy against multidrug-resistant tumors, and the lack of neurotoxicity, D-24851 may have significant potential for the treatment of various malignancies.

INTRODUCTION

Compounds that interfere with the cell cycle have become a major interest in cancer research because they inhibit the proliferation of tumor cell lines derived from various organs (1). The well-characterized and clinically used antimitotic drugs, namely the taxanes (paclitaxel, docetaxel; Refs. 2–5) and the *Vinca* alkaloids (vincristine, vinblastine, vinorelbine; Refs. 6), bind to tubulin, one of the essential proteins for chromosomal segregation. Alternating α- and β-tubulins polymerize to microtubules, long dynamic tubular fibers, which constitute the mitotic spindles. Microtubule inhibitors interfere with the microtubule dynamics of tubulin polymerization and depolymerization, which results in the inhibition of chromosome segregation in mitosis and consequently the inhibition of cell division (7, 8). The three major classes of tubulin-binding agents are the taxanes, which stabilize microtubules by blocking disassembly, the *Vinca* alkaloids, and the colchicine-site binders (9). The latter two are microtubule-destabilizing agents that act by blocking assembly of tubulin heterodimers. A major point is that nondividing cells are extremely resistant toward these drugs (10), whereas uncontrolled dividing tumor cells run into cell cycle arrest by cell cycle checkpoint pathways. Subsequently apoptosis of the cells may be initiated (11).

Although the taxanes and the *Vinca* alkaloids are effective in the treatment of different malignancies, their potential is limited by the development of drug resistance (8). One pathway leading to resistance is mediated by overexpression of transmembrane efflux pumps, namely the p-gp170[4] (12) and the MRP (13). These efflux pumps are able to reduce the intracellular concentrations of taxanes and *Vinca* alkaloids to a nontoxic level. Resistance is also mediated by the expression of tubulin isotypes and mutants that showed impaired taxane-driven tubulin polymerization (14). Another major drawback of taxanes and *Vinca* alkaloids in clinical application is the development of neurotoxicity (15–19). The drugs interfere with the function of microtubules in axons, which mediate the neuronal vesicle transport (8). New chemical entities that bind to tubulin but neither are a substrate of transmembrane pumps nor interfere with the function of axonal microtubules would strongly increase the therapeutic index in the treatment of malignancies. In screening for a compound with these superior characteristics, a molecule named D-24851 was identified that destabilizes microtubules in tumor cells and cell-free systems. D-24851 does not interact with the tubulin-binding sites of vincristine and colchicine. Further characterization revealed that this microtubule inhibitor is not a substrate of p-gp170 nor of MRP and, consequently, retains its antitumoral efficacy in cell lines with MDR or MRP resistance phenotypes. In addition, the administration of D-24851 to rats revealed no deficit in motor function and no change in NCV, which suggested a lack of neurotoxicity of D-24851.

MATERIALS AND METHODS

Materials and Cell lines. D-24851 {*N*-[pyridin-4-yl]-[1-(4-chlorobenzyl)-indol-3-yl]-glyoxylic acid amide; ASTA Medica AG}[5] was synthesized as follows. Reaction of indole with 4-chlorobenzylchloride in dimethylformamide yielded *N*-(4-chlorobenzyl)-indole in 97% yield. Treatment of *N*-(4-chlorobenzyl)-indole with oxalylchloride in *tert*-butylmethylether or diethylether as solvent gave, in 90% yield, 1-(4-chlorobenzyl)-indol-3-yl-glyoxylic acid chloride. Aminolysis reaction of the latter compound with excess of 4-aminopyridine in dimethylformamide under cooling afforded the desired *N*-(pyridin-4-yl)-[1-(4-chlorobenzyl)-indol-3-yl]-glyoxylic acid amide in 64% yield.

General chemicals (paclitaxel, vincristine, vinblastine, and podophyllotoxin) were purchased from Sigma (Munich, Germany). Radiochemicals were obtained from Amersham Corp. P-glycoprotein (C219), and α-tubulin (B-5-1-2) antibodies, were purchased from Alexis Biochemicals Corp. (Grünberg, Germany) and Sigma (Munich, Germany), respectively. Cy3-conjugated and peroxidase-conjugated goat antimouse antibodies were obtained from Dianova (Hamburg, Germany). Tumor cell lines SKOV3 (ovary/human/HTB-77), KB/

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[2] To whom requests for reprints should be addressed, at ASTA Medica AG, Department of Cancer Research-Molecular Biology, Weismuellerstrasse 45, 60314 Frankfurt, Germany. Phone: 49-69-4001-2413; Fax: 49-69-4001-2777.

[3] The abbreviations used are: p-gp170, M_r 170,000 P-glycoprotein; MDR, multidrug resistant; MRP, multidrug resistance protein; NCV, nerve conductance velocity; 5-FU, 5-fluorouracil; XTT, sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis-(4-methoxy-6-nitro)-benzene sulfonic acid hydrate; FACS, fluorescence-activated cell sorting; SRB, sulforhodamine B; ILS, increased life span; GTP, guanosine triphosphate.

[4] Patent no. DE19814838, PCT int. appl. WO 9951224.

HeLa (cervix/human/CCL-17), HT 29 (colon/human/HTB-38), A549 (lung/human/CCL-185), PC-3 (prostate/human/CRL-1435), DU145 (prostate/human/HTB-81), AsPC-1(pancreas/human/CRL-1682), C6 (brain/rat/CCL-107), U 87 (brain/human/HTB-14), MDA-MB 231 (breast/human/HTB-26), and L1210 (leukemia/mouse) were obtained from ATCC. LT12 and LT12/mdr1 were a gift from Dr. K. Nooter (University Hospital Rotterdam, Rotterdam, the Netherlands). The detailed characteristics of human parental A2780/wt (ovarian), MCF-7/wt (breast), HT1080 (fibrosarcoma), HCT-8 (colon), HT-29 (colon) cell lines as well as the MDR p-gp170 overexpressing A2780/Dx5 and MCF-7/adr, the MRP expressing MDR HT1080/Dr4 (20), the cisplatin-resistant A2780/CP2, the 5-FU-resistant HT29-R1 (bolus) and HT29-R24 (continuous exposure), the raltitrexed-resistant HT29/ICID, cell lines have been published previously (21–25).[5]

Flow Cytometry. KB/HeLa cells (1×10^6 cells) were exposed to the cytotoxic agents for 24 h at 37°C, and DNA content of the cells was determined using FACS using a Calibur flow cytometer (Becton Dickinson, Heidelberg, Germany). The Number of cells in G_2-M phase was calculated using Mod Fit LT cell cycle analysis software (VERITY). Data points were connected and IC_{50} calculated using a nonlinear regression program (GraphPad Prism).

Indirect Immunofluorescence Microscopy. KB/HeLa cells were incubated for 24 h with cytotoxic agents and then extracted for 3 min with ice-cold 0.5% Triton X-100 in PHEM containing 10 μM paclitaxel. This treatment removes unassembled tubulin while preserving microtubules (26). The cultures were then fixed by the addition of PHEM containing 8% paraformaldehyde and 0.3% glutaraldehyde at 4°C. Microtubules were visualized using a mouse monoclonal antibody against α-tubulin (Sigma) used at 1:1000 dilution and a Cy3-conjugated goat antimouse antibody used at 1:1000 dilution and a cooled AT200 CCD (charge coupled device) camera system (Photometrics Ltd., Munich, Germany). Fluorescent images were further processed using Fluoro-Pro module for Image-ProPlus.

Tubulin Polymerization Assay. The assay was basically performed according to Bollag *et al.* (27). Microtubules from calf brain (Sigma) were depolymerized according to the manufacturer's protocol. Tubulin heterodimers (10 μM) were incubated with different compounds (1 μM or as indicated) in PEMT buffer (100 mM PIPES, 1 mM EGTA, 1 mM Mg Cl_2, and 0.05% Triton-X-100) containing 1 mM GTP in a total volume of 100 μl at 37°C for 1 h. Triton-X-100 strongly increased the solubility of D-24851 (data not shown). Samples (75 μl) were then transferred to a 96-well Millipore Multiscreen Durapore hydrophilic 0.22-μm pore size filtration plate, which had been previously washed with 200 μl PEM buffer (100 mM PIPES, 1 mM EGTA, and 1 mM Mg Cl_2) under vacuum. Recovered microtubules on the filters were stained with 50 μl of amido black solution (0.1% naphthol blue black (Sigma), 45% methanol, and 10% acetic acid) for 2 min. Vacuum was applied, and unbound dye was removed by two additions of 200 μl of destaining solution (90% methanol and 2% acetic acid). The microtubule bound dye was then eluted by incubation with elution solution (25 mM NaOH, 0.05 mM EDTA, and 50% ethanol) for 10 min. The elution solution was then transferred to a 96-well plate and the absorbance measured at 600 nm.

Spin Column Assay. Tubulin heterodimers (3 μM) were obtained by depolymerization of microtubules purchased from Sigma as described above and were incubated either with 3 μM colchicine containing [^{3}H]colchicine (4×10^4 dpm/nmol) in the presence of D-24851 or podophyllotoxin (A) or with 3 μM vincristine containing [^{3}H]vincristine (4×10^4 dpm/nmol) in the presence of D-24851 or vinblastine (B) in PEMT buffer at 37°C for 1 h. Two aliquots (90 μl) of the incubation mixture (200 μl) were each loaded onto a 0.8-ml Sephadex G25 column previously equilibrated in PEMT. The columns were then placed into 1.5-ml tubes and spun at $200 \times g$ for 1 min, and radioactivity in the flow-through was analyzed by scintillation counting. Radioactivity that was found in the flow-through after centrifugation of the incubation mixture in the absence of tubulin was taken as background. Data points were connected and IC_{50} calculated using a nonlinear regression program (GraphPad Prism).

XTT Assay. The XTT assay (28) was used to determine proliferation by quantification of cellular metabolic activity. Tumor cell lines were cultivated in microtiter plates (1×10^3 cells per well in 100 μl) and were incubated with different concentrations of cytotoxic agents for 48 h. Subsequently, 50 μl of XTT solution (1 mg/ml XTT, 25 μM *N*-methyldibenzopyrazine methyl sulfate) was added per well, and mixtures were incubated for an additional 4 h. The amount of formazan salt was quantified in at least four replicates by absorbance at 490 nm using a Biomek Plate Reader (Beckman). IC_{50}s and inhibition curves connecting the data points were obtained using the nonlinear regression program GraphPad Prism.

SRB Assay. Drug sensitivity was also assessed with the SRB-assay (29). Exponentially growing cells were seeded at a density of 600-1000 cells/well in 96-well microtiter plates (Falcon, Becton Dickinson Labware, Plymouth, United Kingdom) and allowed to attach overnight. After 24 h, cells were exposed for 24 h to either paclitaxel, vincristine, or D-24851, washed twice with PBS, and incubated in drug-free medium. At four cell-doubling times after the beginning of drug treatment, cells were fixed with trichloric acetic acid and were washed and stained with SRB as originally described. The absorbance was measured at 570 nm using a 96-well plate reader (340 EL BIO Kinetics Reader, BIO-TEK Instruments Inc., Winooski, VT). The drug concentrations that inhibited cell growth by 50% (IC_{50}) were determined from semilogarithmic dose-response plots.

L1210 Mouse Leukemia Xenografts. The MDR subline of mouse leukemia L1210 cells (L1210/VCR) was generated by long-term adaptation in a medium with stepwise increasing concentrations of vincristine. Expression of P-glycoprotein was analyzed by Western blotting using monoclonal antibody C219. L1210 and L1210/VCR cells were injected i.p. in male CD 2 F1 mice (20–25 g). The following day, mice were treated with maximally tolerated doses of D-24851 (150 mg/kg p.o.; d1-d4), vincristine (0.5 mg/kg i.p.; d1-d4), paclitaxel (15 mg/kg i.p., d1-d4), or doxorubicin (1 mg/kg i.p.; d1-d4), and the ILS of the mice relative to the vehicle group was monitored. The survival time of the treated animals in percentage (ILS) of the vehicle-treated control group was calculated.

Yoshida AH13 Rat Sarcoma Model. Yoshida AH13 sarcoma cells were implanted i.p. in rats. After two passages, obtained ascites were grafted s.c. into female Sprague Dawley rats (250–290 g). Administration of the compounds or a vehicle control was started when mean tumor weights were approximately 0.5–1 g. D-24851 was given on days 1–5 and 8–12 (10 mg/kg p.o.; d1–5 × 2), paclitaxel was given on days 1–4 and 8–11(2 mg/kg i.p.; d1–4 × 2), and vincristine was given on days 1, 4, 8, and 12 (0.6 mg/kg i.p.; d1, d4 × 2). Body and tumor weights as determined by comparison to the size of standard weights were monitored at day 1, 3, 7, 10, 14, and so forth. All of the studies were conducted in accordance with the local animal ethics regulatory requirements. In accordance with the animal ethics regulations, euthanization of tumor-bearing rats was required when the calculated tumor weight reached ≥10% of the body weight.

Coordination Test (Rota-Rod Test). Rats (Wistar, 250–290 g) were treated with D-24851 (10 mg/kg p.o.; d1–5 × 2), paclitaxel (2 mg/kg i.p.; d1–4 × 2), vincristine (0.4 mg/kg i.p.; d1, d4 × 2), or a vehicle control. Rota-rod testing was performed at day 0, 5, and 10. The rod was set in motion at constant speed (2 rpm), and the rats were placed into individual sections of the apparatus. The rod was then accelerated from the rate of 2 rpm to 6 rpm. The animal's performance was recorded as the time that had elapsed when the rats fell off the rotating rod. The data were analyzed using 1-way ANOVA, followed by Newmann-Keuls test as appropriate. All of the results are expressed as mean ± SE with $n = 6$ animals. All of the studies were conducted in accordance with the local animal ethics regulatory requirements.

NCV. Rats were treated with the compounds as described above in the section on "Coordination Test." At days 0, 5, and 10, the NCV was recorded in accordance with the method as described previously (30). The equipment used were the electrical stimulator ESU-2, amplifier of biological potentials, oscillograph, and photorecorder. The temperature in the room during the experiments was kept at the level of 28–30°C. NCV was measured retrospectively using the photos. The data were analyzed using 1-way ANOVA, followed by Newmann-Keuls test as appropriate. All of the results are expressed as mean ± SE with $n = 6$ animals. All of the studies were conducted in accordance with the local animal ethics regulatory requirements.

RESULTS

Effect of D-24851 on Cell Cycle. D-24851 (chemical structure see Fig. 1*A*) was identified in a cytotoxic assay and first evaluated by cell

[5] Experiments with HT1080/Dr4 cells were performed in cooperation with Dr. Y. M. Rustum, Roswell Park Cancer Institute, Buffalo, NY.

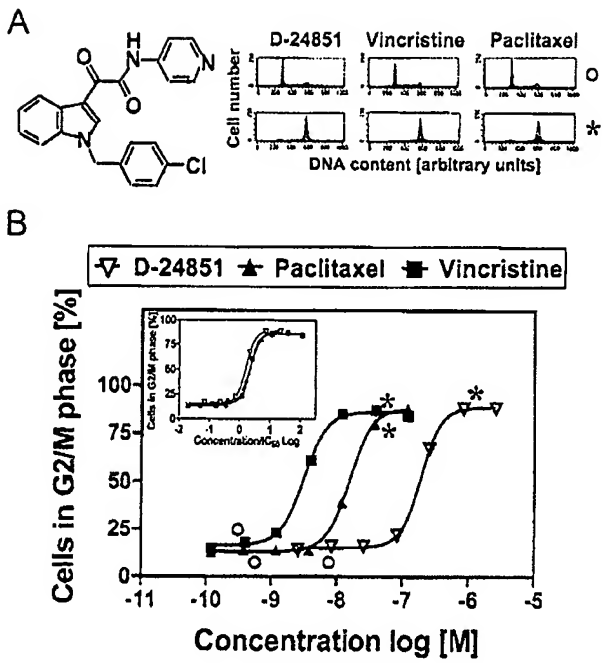

Fig. 1. Effect of D-24851 on cell cycle. KB/HeLa cells were treated with different concentrations of D-24851 (chemical structure, *A*, *left panel*), vincristine, or paclitaxel for 24 h, and DNA content of the cells was analyzed by FACS. *A*, *right panel*, *upper diagrams*, treatment with low concentrations of the compounds (O); *lower diagrams*, treatment with higher concentrations of the compounds (*). In *B*, percentage of cells in G_2-M phase is plotted against the concentrations of the compounds. O, data points correspond to the lower diagrams in *A*; *, data points correspond to the upper diagrams in *A*. *Inset*, percentage of cells in G_2-M phase is plotted against the ratio of the concentrations of the compounds relative to their IC_{50}s. Data points were connected using a nonlinear regression program. Single experiments from at least two independent experiments are shown.

cycle analysis using flow cytometry. KB/HeLa cells were exposed to different concentrations of D-24851 for 24 h. At low concentration of D-24851 (8.5 nM), the main part of the cells were found at G_1 phase of the cell cycle as observed with the solvent control, which indicated that the compound causes no detectable cell cycle effect at this concentration (Fig. 1*A*, *upper diagram*, peak at 300). At higher concentrations of D-24851 (850 nM), a complete shift from G_1 to G_2-M phase was observed (Fig. 1*A*, *lower diagram*, peak at 600). Thus, D-24851 induces an accumulation of KB/HeLa cells specifically in G_2-M phase of the cell cycle. To compare D-24851 with the known G_2-M cell cycle inhibitors vincristine and paclitaxel, we treated KB/HeLa cells with different concentrations of the compounds (Fig. 1*A*). The cells also completely shifted from G_1 to G_2-M phase (Fig. 1*A*, *upper* and *lower diagrams*, respectively). When the percentage of cells in G_2-M phase were plotted against different concentrations of the compounds, D-24851, vincristine, and paclitaxel arrested the cell cycle in a concentration-dependent manner with IC_{50} values of 190, 3, and 15 nM, respectively (Fig. 1*B*). When the percentage of cells accumulated in G_2-M phase were plotted against the concentration divided by their IC_{50} values of cell growth inhibition (Table 1), the resulting curves were nearly identical (Fig. 1*B*, *inset*). This indicates that the cell cycle arrest of all of the compounds correlates with the cell growth inhibition and that D-24851 may have a mode of action similar to that of paclitaxel and vincristine.

Effect of D-24851 on Microtubule Organization in Mitotic Spindles. To test whether D-24851 affects the microtubule organization in mitotic spindles, we treated human KB/HeLa cells or human SKOV3 ovarian carcinoma cells with D-24851 for 24 h and subsequently extracted the cells with Triton X-100 and paclitaxel. This treatment removes unassembled tubulin while preserving microtubules (24). Microtubules were then visualized by indirect immunofluorescence using an antibody against α-tubulin. Fig. 2, *A* and *B*, show solvent-treated (control) cells with microtubules constituting the cell shape. In contrast, microtubules were mainly found organized in mitotic spindles with abnormal structures after D-24851 treatment (45 or 80 nM) of the cells (Fig. 2, *C* and *D*). The abnormal spindles had extremely long astral microtubules and were seen monopolar or bipolar. When cells were exposed to higher concentrations of D-24851 (400 or 450 nM), we observed fragmented mitotic spindles (Fig. 2, *E* and *F*). This indicates that D-24851 destabilizes the microtubules of mitotic spindles in a concentration-dependent manner. Similar effects on tumor cells were observed by exposure to vincristine (1–15 nM; Ref. 31), a known microtubule-destabilizing agent (Fig. 2, *G–J*). Microtubules were also found fragmented over the entire cell (Fig. 2, *I* and *J*). Treatment of KB/HeLa cells with paclitaxel (3 or 15 nM) revealed mitotic cells with at least three regions of accumulated microtubules (Fig. 2*K*). These cells could have more than two mitotic spindles. SKOV3 cells revealed microtubules organized in star-like structures that could represent monopolar spindles after paclitaxel treatment (Fig. 2*L*). In contrast to D-24851 and vincristine, fragmentation of microtubules could not be observed in paclitaxel-treated cells. These data are consistent with the stabilizing effect of paclitaxel on microtubules (9).

Effect of D-24851 on Polymerization of Purified Tubulin. To investigate whether D-24851 induces destabilization of microtubules in a cell-free system, purified tubulin was allowed to polymerize in the absence or presence of different compounds. Microtubule polymers were separated from tubulin heterodimers by filtration as described previously (26). When paclitaxel (1 μM) was added to the assay, the amount of microtubule protein recovered was strongly increased as compared with the solvent (DMSO) alone (Fig. 3*A*, *Lane 2 versus Lane 1*). In contrast, no microtubule polymers could be recovered after the addition of vinblastine (1 μM) to the assay (Fig. 3*A*, *Lane 3*). This is consistent with the known destabilizing effect of vinblastine on microtubules (7, 8). D-24851 also completely blocked tubulin polymerization at a concentration of 1 μM (Fig. 3*A*, *Lane 4*). D-25552, a derivative of D-24851 that was not found to be active in our initial screening assay had no effect on tubulin polymerization (Fig. 3*A*, *Lane 5*). These data suggest that D-24851 binds directly to tubulin and thereby inhibits polymerization of tubulin. To compare the activity of vincristine and D-24851 on the inhibition of tubulin polymerization, different concentrations of the compounds were used in the assay. As shown in Fig. 3*B*, D-24851 blocked tubulin polymerization in a

Table 1 *Cytotoxic activity of D-24851 against different tumor cell lines*

All of the experiments were performed in at least four replicates using the XTT assay as described in "Materials and Methods." IC_{50}s were calculated using a nonlinear regression program.

Tumor cell line (tissue/species)	Growth inhibition constant (IC_{50}) [μM]		
	D-24851	Paclitaxel	Vincristine
SKOV3 (ovary/human)	0.036	0.007	0.002
KB/HeLa (cervix/human)	0.115	0.007	0.001
HT 29 (colon/human)	0.072	0.010	0.005
A549 (lung/human)	0.164	0.013	0.027
PC-3 (prostate/human)	0.064	0.012	0.004
DU145 (prostate/human)	0.148	0.012	0.010
AsPC-1 (pancreas/human)	0.285	0.012	0.017
C6 (brain/rat)	0.200	0.047	0.007
U 87 (brain/human)	0.077	0.013	0.003
MDA-MB 231 (breast/human)	0.074	0.011	0.008
L1210 (leukemia/mouse)	0.089	0.140	0.021

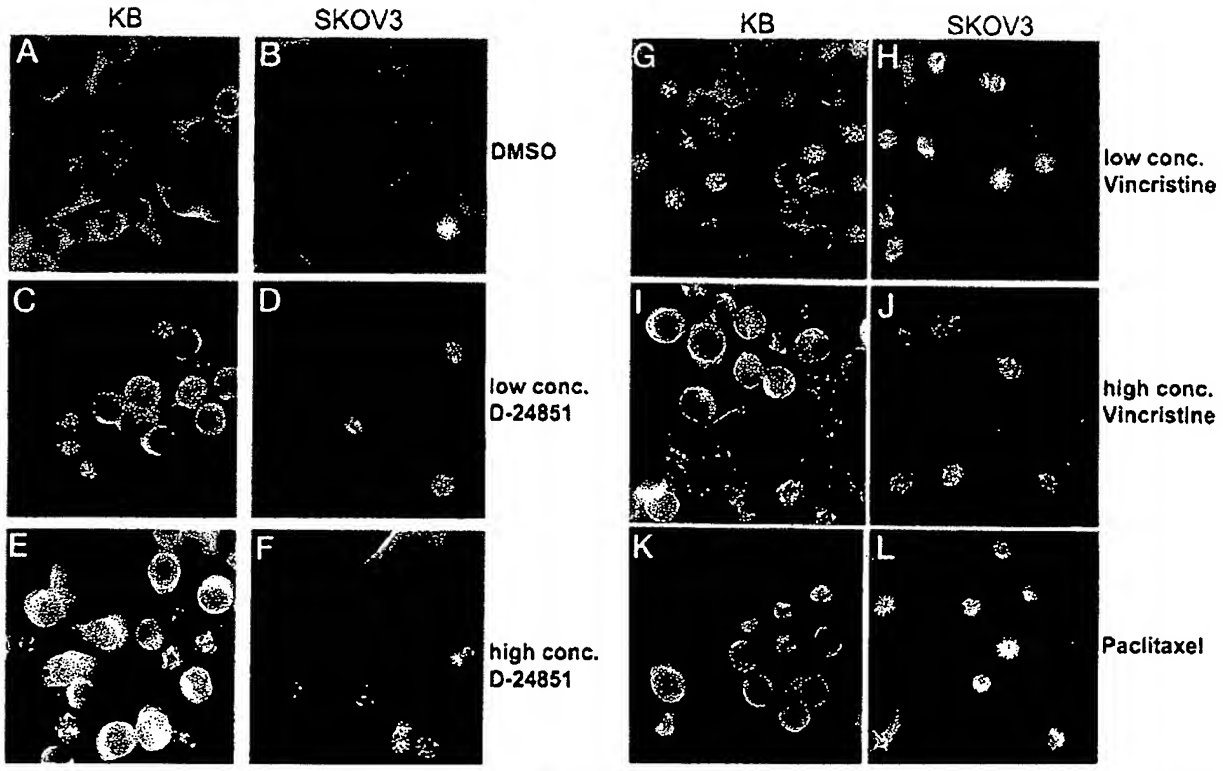

Fig. 2. Effect of D-24851 on stability of microtubules. Treatment of KB/HeLa and SKOV3 cells with DMSO was used as a control (*A* and *B*, respectively). KB/HeLa were exposed to 80 nM (*C*) or 400 nM (*E*) D-24851, 1 nM (*G*) or 10 nM (*I*) vincristine, and 3 nM paclitaxel (*K*). SKOV3 cells were exposed to 45 nM (*D*) or 450 nM (*F*) D-24851, or 3 nM (*H*) or 15 nM (*J*) vincristine, or 15 nM paclitaxel (L). After 24-h exposure to the compounds, microtubules were visualized by indirect immunofluorescence microscopy using an antibody against α-tubulin (B5-1-2).

concentration-dependent manner with a IC_{50} of 0.3 μM, whereas the IC_{50} value of vincristine was 10-fold lower.

Competition of Binding of Vincristine or Colchicine to Tubulin by D-24851. The microtubule destabilizing agents vincristine and colchicine are known to bind to tubulin at different sites. To address whether D-24851 binds to tubulin at one of those sites, binding of radiolabeled colchicine or vincristine to tubulin was tested in the presence or absence of unlabeled D-24851 using a spin column assay (32). Free radiolabeled compounds were retained in the column, whereas tubulin-bound compounds were found in the flow-through. When tubulin was incubated with [^{3}H]colchicine in the presence of different concentrations of unlabeled podophyllotoxin, a known competitor of colchicine binding to tubulin, and subsequently spun through the column, the amount of [^{3}H]colchicine found in the flow-through was strongly reduced (Fig. 4*A*). Thus, podophyllotoxin competes for the binding of colchicine to tubulin. In contrast, when different concentrations of D-24851 instead of podophyllotoxin were used in the assay, no effect on binding of [^{3}H]colchicine to tubulin was observed (Fig. 4*A*). Thus, D-24851 does not bind to the colchicine-binding site of tubulin. To test whether D-24851 might overlap with the vincristine binding site of tubulin, [^{3}H]vincristine was incubated with tubulin in the presence of the unlabeled vincristine derivative vinblastine or unlabeled D-24851. As shown in Fig. 4*B*, increasing concentrations of vinblastine reduced the binding of [^{3}H]vincristine to tubulin. In contrast, D-24851 was not able to compete for [^{3}H]vincristine binding to tubulin up to a concentration of 100 μM. This indicates that D-24851 does also not bind to the vincristine-binding site of tubulin to induce disassembly of microtubules.

Effect of D-24851 on Growth of Different Tumor Cell Lines. To explore the effect of D-24851 on tumor cell growth we treated human and rodent tumor cell lines from prostate, brain, breast, pancreas, colon, lung, ovary, and cervix with different concentrations of D-24851, paclitaxel, or vincristine. Cytotoxicity was measured by cellular metabolic activity using the XTT assay. Growth of all cell lines was inhibited by all three compounds in a concentration-dependent manner. The growth inhibition constants (IC_{50}) of the different tumor cell lines ranged from 0.002 to 0.027 μM for vincristine, from 0.007 to 0.047 μM for paclitaxel, and from 0.036 to 0.285 μM for D-24851 (Table 1). This indicates that D-24851 inhibits growth of various human tumor cell lines at 10- to 20-fold higher concentrations than those of paclitaxel and vincristine.

Cytotoxic Activity of D-24851 on MDR Tumor Cell Lines. One major mechanism of multidrug resistance is mediated by the overexpression of the p-gp170 (8). The antitumoral efficacy of D-24851 was compared with vincristine, paclitaxel, and doxorubicin in the vincristine-selected MDR mouse leukemia cell line L1210/VCR using the cytotoxicity assay. Overexpression of p-gp170 in L1210/VCR was confirmed by Western blot analysis using monoclonal antibody C219 (Ref. 33; Fig. 5*A*).

The resistant factors (RFs) as determined by the ratio of the growth inhibition constants (IC_{50}) of the resistant cell line relative to those of its parental cell line were ~1 for D-24851 and >56 for the other three anticancer agents (Fig. 5*B*; Table 2). Thus, L1210/VCR cells were resistant to vincristine, paclitaxel, and doxorubicin, whereas no cross-resistance to D-24851 was observed. In addition, three other p-gp170-overexpressing cell lines, including the mdr1 cDNA-transfected acute myeloid leukemic rat cell line LT12, were tested. In all cases, no

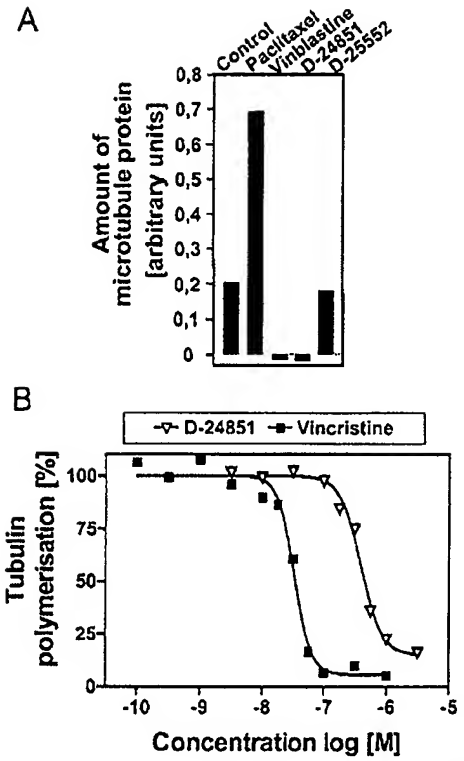

Fig. 3. Effect of D-24851 on polymerization of purified tubulin. Purified bovine brain tubulin was incubated with GTP in the presence of DMSO alone as a control or different compounds dissolved in DMSO as indicated (*A*) or with different concentrations of D-24851 or vincristine (*B*). Polymerized microtubules were separated from heterodimeric tubulin by filtration and recovered on 0.22-μm pore size filters in a 96-well plate. Subsequently, microtubules were stained with naphthol blue black, and the amount of the dye was quantified. Data points are the means of duplicates and were connected using a nonlinear regression program. A single experiment of three independent experiments with similar results is shown.

cross-resistance of D-24851 to vincristine or paclitaxel was observed (resistance factors <2), whereas up to 1000-fold resistance to vincristine or paclitaxel was found (Table 2). Thus, in contrast to paclitaxel and vincristine, the cytotoxic efficacy of D-24851 against tumor cells is not altered by the MDR1 phenotype. In addition, the antitumoral efficacy of D-24851 was evaluated in resistance mediated by the MRP and in human tumor cells with resistance to cisplatin, the topoisomerase-I-inhibitor SN-38 (7-ethyl-10-hydroxycamptothecin), and thymidylate synthase inhibitors (*e.g.*, 5-FU and raltitrexed). Taken together, D-24851 retained unaltered cytotoxic efficacy toward all of the resistant sublines tested (Table 2). To test the cytotoxic efficacy of D-24851 toward MDR tumor cells *in vivo*, we used the L1210 leukemic mouse model. L1210 or L1210/VCR cells were implanted i.p. into mice. The mice were then treated with maximal tolerated doses (daily-times-four schedule) of D-24851, vincristine, paclitaxel, or doxorubicin and ILS of the mice relative to the vehicle control group was monitored. As shown in Fig. 5*C*, all of the three compounds were able to increase life span of mice grafted with parental L1210 cells (*open bars*). In contrast, only D-24851 was able to increase life span of the mice grafted with resistant L1210/VCR cells (*closed bars*). Moreover, the antitumoral efficacy of D-24851 was equal in the L1210 and L1210/VCR mouse model. Thus, the overexpression of P-glycoprotein in tumor cells does not influence the antitumoral *in vivo* efficacy of D-24851.

***In Vivo* Efficacy of D-24851 in Rat Yoshida AH13 Sarcoma Model.** D-24851 affects the *in vitro* growth of different rodent and human tumor cell lines. To test whether D-24851 also affects growth of solid tumors *in vivo*, we used the Yoshida AH13 rat sarcoma model. Exponentially growing AH13 sarcoma tumor cells were s.c. grafted into rats. When tumors reached an initial weight of ~0.5–1 g, animals were treated with D-24851 (10 mg/kg p.o.; day 1–5 for 2 weeks) and maximal tolerated doses of paclitaxel (2 mg/kg i.p.; d1–4 × 2; Fig. 6B) and vincristine (0.6 mg/kg i.p.; d1, d4 × 2; Fig. 6B). D-24851 doses of the selected schedule exerted the maximal antitumoral efficacy and were ~1% of the acute toxicity LD_{50}. Single D-24851 doses showed no antitumoral efficacy. The acute toxicity LD_{50} of p.o.D-24851 (single doses) to rats were higher than 850 mg/kg. As shown in Fig. 6*A*, D-24851 induced complete tumor remissions (cures) of animals. Curative doses of D-24851 were well tolerated with low or no systemic toxicity as indicated by an increase in body weight (Fig. 6*B*). Preliminary data also indicate that D-24851 exerts no hematological toxicities(data not shown). In contrast to D-24851, vincristine or paclitaxel administration resulted only in a modest inhibition of tumor growth when compared with the vehicle control group (Fig. 6*A*). The inhibition of tumor growth by vincristine or paclitaxel could be observed only when maximal tolerated (toxic) doses were administered coinciding with a loss of body weight >10% of the initial body weight (Fig. 6*B*). Thus, only D-24851 exerts potent antitumoral efficacy toward this solid tumor with low systemic toxicity.

Preliminary data on nude mice show that D-24851 also inhibits tumor growth in human xenografts, *e.g.*, s.c. transplanted PL-3 prostate carcinoma.

Neurotoxicity. Administration of paclitaxel and vincristine is associated with a number of toxic side effects (16). One of the dose-

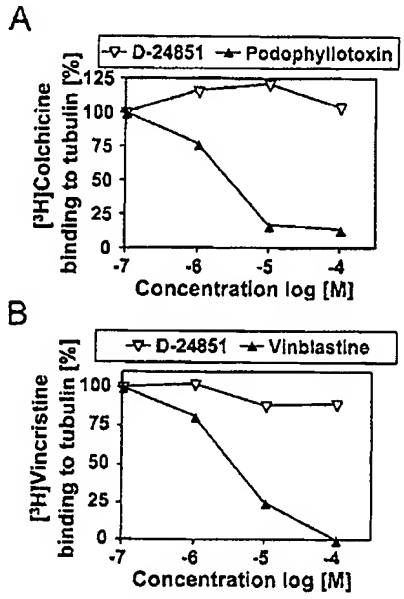

Fig. 4. Competition of D-24851 for binding of [^{3}H]colchicine or [^{3}H]vincristine to tubulin. In *A*, [^{3}H]colchicine was incubated with tubulin in the presence of different concentrations of podophyllotoxin or D-24851. In *B*, [^{3}H]vincristine was incubated with tubulin in the presence of different concentrations of vinblastine or D-24851. Incubation mixtures were centrifuged through Sephadex G25 columns, and radioactivity in the flow-through was measured by scintillation counting as described in "Materials and Methods." Tubulin-bound [^{3}H]colchicine and [^{3}H]vincristine were plotted against the concentrations of the competitors. Data points are the means of duplicates and represent a single experiment of at least three independent experiments with similar results.

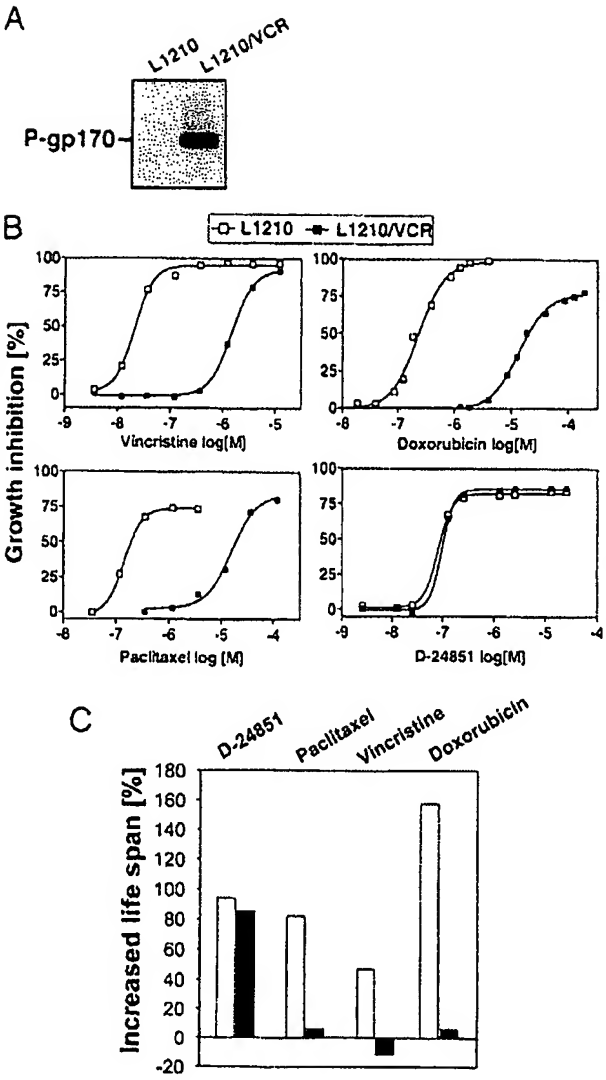

Fig. 5. Effect of D-24851 on growth of MDR cell lines *in vitro*. In *A*, L1210 and L1210/VCR cells were lysed, and proteins were subjected to SDS-PAGE and Western blot analysis using a monoclonal antibody specific for P-glycoprotein (C217). In *B*, L1210 and L1210/VCR were exposed to different concentrations of the antimitotic agents. Inhibition of cell growth was then analyzed by the XTT assay. The curves were fitted to the data points (means of at least four replicates) using a nonlinear regression program. *C*, L1210 mouse leukemia *in vivo* model. L1210 cells (*white bars*) and L1210/VCR cells (*black bars*) were implanted i.p. into mice. Mice were subsequently treated four times with maximally tolerated doses of D-24851 (150 mg/kg p.o.; day 1–4), vincristine (0.5 mg/kg; i.p.; d1–d4), paclitaxel (15 mg/kg i.p.; d1–d4), and doxorubicin (1 mg/kg; i.p.; d1–d4). ILS of the mice in percentage relative to the vehicle-control group were determined.

limiting side effects appears to be neuronal damage as elicited in motor as well as sensory deficits (34). The effect of D-24851 or vincristine on motor function of rats was tested using a coordination test (Rota-rod testing). To this end, rats were treated with the compounds and subsequently placed on a rotating rod. At days 5 and 10 the performance of the animal was monitored as the time that had elapsed when the rat fell off the rod. When rats were dosed with D-24851 (10 mg/kg p.o.; day 1–5 for 2 weeks) no significant difference in their performance on the accelerating Rota-rod treadmill from that of the control group was observed (Fig. 7*A*). In contrast, the performance of rats receiving paclitaxel (2 mg/kg i.p.; d1–4 × 2) or vincristine (0.4 mg/kg i.p.; d1, d4 × 2) strongly decreased (Fig. 7*A*). This suggests that D-24851 administration to rats revealed no deficit in motor function in contrast to paclitaxel or vincristine at antitumoral efficacious doses.

The determination of peripheral NCV might also serve as a reliable index of neurotoxicity (35). To this end, the effect of D-24851 in comparison with paclitaxel and vincristine on the NCV in rat tail was investigated at days 5 and 10 after the onset of treatment. When rats were dosed with D-24851 (10 mg/kg p.o.; d1–5 × 2) no change in NCV was observed as shown for the control group receiving only the vehicle (Fig. 7*B*). Treatment of rats with vincristine (0.6 mg/kg i.p.; d1, d4 × 2) or paclitaxel (2 mg/kg i.p.; d1–4 × 2) resulted in a strong decrease of NCV, which has been also reported previously (Fig. 7*B*; Ref. 18, 34). NCV of rats that have been treated with anticancer compounds strongly correlated with the performance of the rats on the rotating rod as well as with the body weight of the animals (Fig. 7*C*). These data demonstrate that D-24851 shows no neurotoxicity at antitumoral efficacious doses *in vivo*.

DISCUSSION

D-24851 is a novel synthetic anticancer agent with significant antitumoral activity *in vitro* and *in vivo*. It destabilizes microtubules in tumor cells as well as in a cell-free system. The binding site of D-24851 does not overlap with the tubulin-binding sites of the well-characterized microtubule destabilizing agents vincristine or colchicine. Furthermore, the molecule selectively blocks cell cycle progression at metaphase. *In vitro*, D-24851 exerts significant antitumoral activity against a variety of malignancies (*e.g.*, prostate, brain, breast, pancreas, and colon). When compared with other microtubule-inhibiting compounds, D-24851 has a number of superior properties *in vivo*: (*a*) curative treatment of Yoshida AH13 rat sarcomas at almost nontoxic doses; (*b*) oral applicability; (*c*) lack of neurotoxicity at curative doses, which is a major drawback of taxanes and *Vinca* alkaloids in the clinical use; and (*d*) efficacy toward MDR tumor cells. Therefore, D-24851 may have significant potential as a therapeutic agent in cancer therapy.

The mode of action of the molecule as a tubulin inhibitor was shown by indirect immunofluorescence microscopy using an antibody against α-tubulin and in a cell-free tubulin polymerization assay.

Table 2 *Antitumoral efficacy of D-24851 against tumor cell lines with different resistance phenotypes*

All of the experiments were performed at least in triplicates using cytotoxic assays (XTT or SRB assay) as described in "Materials and Methods."

		Growth inhibition constant [μM] (rF)[a]		
Resistance type	Cell line	D-24851	Vincristine	Paclitaxel
MDR1	L1210 (parental)	0.089	0.021	0.14
	L1210/VCR	0.080 (0.9)	1.575 (75)	15.7 (110)
MDR1	LT12 (parental)	0.035	0.001	0.012
	LT12/mdr1	0.042 (1.2)	0.014 (14)	0.22 (18)
MDR1	MCF-7 (parental)	0.057	0.001	0.003
	MCF-7/adr	0.083 (1.5)	1.2 (1200)	2.1 (700)
MDR1	A2780 (parental)	0.026	0.0006	0.005
	A2780/Dx5	0.041 (1.6)	0.024 (40)	0.145 (29)
MRP	HT1080 (parental)	0.031	0.0008	0.003
	HT1080/DR4	0.030 (1.0)	0.018 (23)	0.005 (1.7)
Cisplatin	A2780 (parental)	0.026	0.0006	0.005
	A2780/CP2	0.048 (1.9)	0.003 (4)	0.007 (1.4)
5-FU	HT29 (parental)	0.059	0.003	0.007
Bolus	HT29-R1	0.057 (1.0)	0.002 (0.7)	0.006 (0.9)
Continuous	HT29-R24	0.065 (1.1)	0.003 (1)	0.006 (0.9)
Raltitrexed	HT29/ICID	0.064 (1.1)	0.006 (2)	0.016 (2.2)
Topoisomerase-I	HT29/SN38	0.070 (1.2)	0.003 (0.9)	0.008 (1.1)
Topoisomerase-I	HCT-8 (parental)	0.037	0.013	0.033
	HCT-8/SN38	0.037 (1.0)	0.020 (1.5)	0.133 (4.0)

[a] RF, resistance factor was calculated from the ratio of the growth inhibition constant (IC_{50}) for the resistant cell subline and that for their parental cell line.

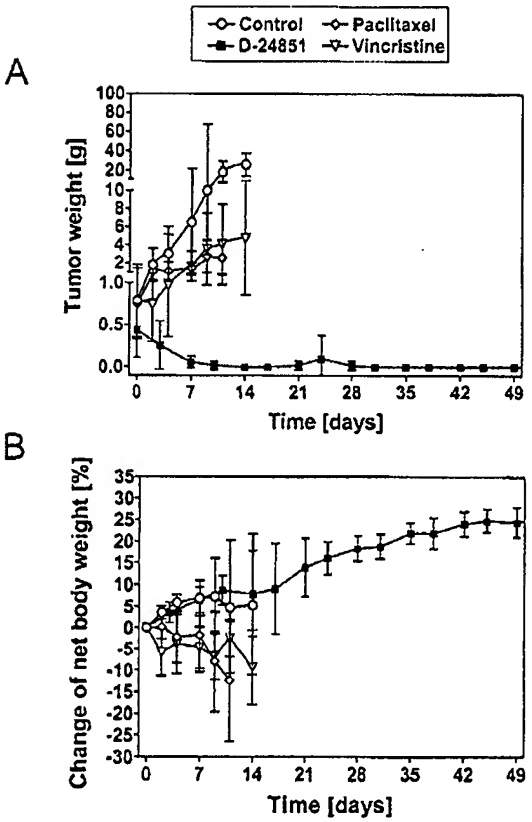

Fig. 6. Efficacy of D-24851 toward Yoshida AH13 rat sarcomas. Tumor cells were implanted s.c. in rats. Administration of D-24851 (10 mg/kg p.o.; d1–5 × 2), paclitaxel (2 mg/kg i.p.; d1–4 × 2), vincristine (0.6 mg/kg i.p.; d1, d4 × 2), or a vehicle control was started when mean tumor weights were ~0.5–1 g. Change of tumor weights (*A*) and net body weights (*B*) were plotted against time and are the means ± SD from seven animals. A representative experiment of at least six independently performed experiments is shown.

D-24851 induced accumulation of cells with condensed nuclei (data not shown) and abnormal mitotic spindles. At higher concentrations, fragmentation of the spindle apparatus and degradation of microtubules were observed. The well-characterized *Vinca* alkaloids and colchicine interact with different binding sites on tubulin and were known to destabilize microtubules (7). In fact, exposure of cells to vincristine also revealed fragmented mitotic spindles similar to those shown for D-24851. Paclitaxel, known as a microtubule stabilizing agent, did not induce fragmentation of the spindle apparatus. This strongly suggests that D-24851 arrests cells at metaphase because of modulating microtubule stability.

The destabilizing effect of D-24851 on microtubules was also seen in a cell-free assay using purified tubulin. Polymerization of tubulin was blocked by D-24851 in a concentration-dependent manner with an IC_{50} of approximately 0.3 μM, which may indicate a direct interaction of D-24851 with tubulin. The substoichiometric concentrations of the compound in relation to the tubulin concentration (10 μM) are sufficient to block tubulin polymerization, similar to vincristine or other *Vinca* alkaloids (36).

D-24851 is a low-molecular-weight compound that shows no structural similarities to *Vinca* alkaloids or colchicine and did not compete for the binding of radiolabeled vincristine or colchicine to tubulin. This suggests that D-24851 may bind to a novel binding site on tubulin that results in inhibition of tubulin polymerization.

The IC_{50} value of vincristine for tubulin polymerization was ~10-fold lower than that of D-24851. The difference between both compounds in the inhibition of tubulin polymerization was also observed in inducing cell cycle arrest and in the inhibition of cell growth in a variety of different tumor cell lines. This suggests that D-24851 interferes with the function of tubulin, thereby inducing cell cycle arrest and consequently cell growth inhibition.

In vivo, D-24851 showed a remarkable antitumoral efficacy in the Yoshida AH13 rat sarcoma model. Oral application of D-24851 induced complete tumor regressions and resulted in curative treatment of the animals. Of great importance is that, at curative doses of D-24851, no systemic toxicity in terms of body weight loss or hematological toxicities were observed *in vivo*. In contrast, vincristine or paclitaxel treatment at their maximal tolerated doses resulted only in a moderate inhibition of tumor growth but in significant toxicity in terms of body weight loss. These data demonstrate that D-24851 is more potent than vincristine or paclitaxel in the treatment of Yoshida AH13 tumors *in vivo*.

In clinical studies it has been demonstrated that cumulative doses of paclitaxel or vincristine doses are associated with development of

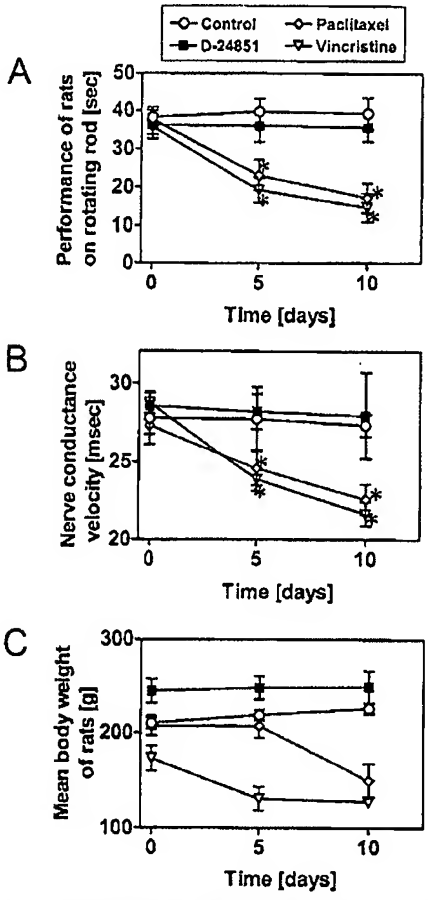

Fig. 7. Neurotoxic effects of antimitotic agents. Rats were dosed with D-24851 (10 mg/kg p.o.; day 1–5 for 2 weeks), paclitaxel (2 mg/kg i.p.; day 1–4 for 2 weeks), vincristine (0.4 mg/kg i.p.; d1, d4 × 2), or a vehicle control, and neurotoxic effects were monitored at days 0, 5, and 10. *A*, Rota-rod testing. Rats were placed on a accelerating rotating rod and the time monitored that had elapsed when the rats fell off the rod. *B*, NCV. Difference of NCV in rat tail was measured as described in "Materials and Methods." *C*, body weight of the animals during treatment with the compounds. Performance of rats on rotating rod, NCV, and body weight are the means ± SE from at least six animals. *, significant difference from control group.

neurotoxicity (15, 16). The effect of these drugs on the nervous system of rats has also been shown previously (19, 35). i.v. administration of *Vinca* alkaloids significantly impaired coordination and NCV in the nerve tail. We also observed similar effects on rats after i.p. application of paclitaxel or vincristine. On a molecular level, drug-impaired microtubule function in axons seems to be responsible for the neurotoxic effects (8). Microtubules were found to accumulate in axons after the administration of paclitaxel, whereas *Vinca* alkaloids interfere with axonal transport, which induces spiralization of axonal microtubules. Although D-24851 also alters microtubule function, no neurotoxic effects on rats in terms of deficit in motor function or reduced NCV was seen at curative doses. One possible explanation for the lack of neurotoxicity of D-24851 could be that concentrations of D-24851 that are sufficient to block the cell cycle do not inhibit axonal vesicle transport. Alternatively, D-24851 may only interact with nonaxonal microtubules.

The use of cytotoxic agents is often accompanied by development of MDR tumor phenotype. A major determinant of MDR is the overexpression of drug efflux pumps, namely the p-gp170 and the MRP. The results reported herein suggest that D-24851 is a substrate neither of P-glycoprotein nor for MRP. Thus, D-24851 retains its cytotoxic activity toward MDR cells *in vitro* and *in vivo*. In contrast, paclitaxel and vincristine were shown to be actively transported by p-gp170 and, in part, by MRP. Of clinical importance is that D-24851 retains its antitumoral activity against cancer cell lines with resistance to cisplatin, the topoisomerase-I-inhibitor SN-38, and the thymidylate synthase inhibitors 5-FU and raltitrexed.

In summary, D-24851 is a novel tubulin-binding agent with significant antitumoral efficacy *in vitro* and *in vivo*. The lack of neurotoxicity and the potential in an oral formulation may provide an anticancer drug with a significant therapeutic index. Clinical Phase I trials with D-24851 will be initiated.

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Resistance to Multiple Drugs

De novo and acquired cross-resistance to multiple antineoplastic agents can result from several alternative factors and processes. Accordingly, we have grouped the major patterns of cross-resistance into several categories, on the basis of their presumed underlying mechanisms (Table 48-2). First, MDR patterns of cross-resistance are frequently associated with decreased drug accumulation, usually because of increased drug efflux. Classic MDR associated with resistance to drugs listed in Table 48-3 is mediated by P-glycoprotein (MDR1, P-170). More recently, a similar but distinct MDR phenotype was attributed to the energy-dependent drug efflux activities of multidrug resistance protein (MRP) family members.[33–36] Another overlapping but discrete resistance MDR phenotype is associated with increased expression of the recently isolated putative efflux transporter, breast cancer resistance protein (BCRP). [37, 38] MDR has also been described in association with overexpression of the lung resistance protein (LRP). The mechanism of LRP-associated resistance is unclear, and whether LRP alone is sufficient to confer resistance is unknown. It is speculated that as a major vault protein, LRP is involved in nucleocytoplasmic transport and may be able to prevent entry of drugs into the nucleus. [39, 40] Drug resistance defined by alterations in topoisomerases represents a third major category of MDR.[26–32] Additionally, more speculative mechanisms of MDR mediated by nonspecific xenobiotic metabolizing enzymes, and cell-to-cell transfer of genetic information are discussed separately. As discussed below, there can be overlap among some of these mechanisms—for example, a high-level resistance to some drugs may depend on expression of both the Phase II drug conjugating glutathione/glutathione *S*-transferase system and the MRP1 glutathione conjugate transporter. [41]

Classic (P-Glycoprotein-Dependent) MDR

An in vitro model of MDR was described by Biedler and coworkers three decades ago. [42] In these studies, cultured cells selected for resistance by exposure to actinomycin D developed cross-resistance to a surprising array of structurally diverse compounds, including vinca alkaloids, puromycin, daunomycin, and mitomycin C. Subsequently, induction of this pattern of cross-resistance has been observed by numerous investigators, who have selected cells in the presence of the same and other drugs. Generally,

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exposure of cells to any of the drugs (many of which are listed in Table 48-3) related to this MDR phenotype can result in cross-resistance to all other members of the phenotype. [8, 9] Drug transport studies using parental and MDR cells demonstrate that the reduced cytotoxicity of these drugs is the result of decreased drug accumulation secondary to enhanced drug efflux. [43, 44] Furthermore, the emergence of MDR has been associated with increased levels of a membrane-bound glycoprotein, P-glycoprotein (P-170 or MDR1 protein).

Although it is widely accepted that P-glycoprotein mediates an energy-dependent decrease in drug accumulation, there is considerable debate on the precise mechanism(s) involved. Drugs associated with the classic MDR phenotype are generally freely permeable to the plasma membrane. In one model, drugs in the cytosol may be recognized by P-glycoprotein and exported back across the plasma membrane in association with adenosine triphosphate (ATP) hydrolysis. [9] Another proposal, termed the "hydrophobic vacuum cleaner" model, suggests that the lipid-soluble drugs may be recognized by P-glycoprotein within the plasma membrane and expelled without ever entering the cytoplasm. [9, 45] A third model is the lipid "flippase" model, in which the role of P-glycoprotein is to merely flip drugs from the inner leaflet of the cell membrane to the outer leaflet, and therefore drive diffusion of drug out of the cell. [46] Numerous other mechanisms of P-glycoprotein-dependent drug transport have been proposed, including processes in which drug efflux is indirectly influenced by P-glycoprotein-mediated changes in membrane potential or chloride channels. [47–50] Finally, it has been noted that if the freely diffusible, lipid-soluble drugs are the substrates of P-glycoprotein, then huge expenditures of energy would be required to maintain reduced drug accumulation in cells continually exposed to extracellular drug. To obviate this thermodynamic obstacle, it has been suggested that the true substrates of P-glycoprotein-mediated efflux may not be the parent drugs, but rather the previously unidentified amphiphilic and membrane-impermeable drug conjugates formed within the cell. [51]

Regardless of the mechanistic details, a great deal of evidence supports the consensus view that P-glycoprotein is the energydependent drug efflux pump responsible for MDR. First, gene transfer experiments show that the expression of P-glycoprotein genes is sufficient to confer drug resistance. [52, 53] Second, P-glycoproteinbelongs to a multigene family of transport proteins (ABC transporters), all of which share sequence homology with several bacterial transport proteins. [54, 55] Third, photoaffinity labeling experiments demonstrate direct binding of drugs to P-glycoprotein. [56] Finally, the distribution of P-glycoprotein on the luminal surfaces of normal tissues including renal tubules, colon, small intestine, and bile canaliculi is consistent with its proposed role in excretory transport. [57] Thus, P-glycoprotein appears to fulfill the requirements predicted of a membrane-bound energy-dependent drug exporter. Although there are two human *MDR* genes, only *MDR1* confers drug resistance. [8, 9]

P-glycoprotein-associated MDR displays significant phenotypic heterogeneity. The relative degree of cross-resistance to the drugs listed in Table 48-3 varies based on the cell line and the selecting drug. While the level of drug resistance is roughly correlated with the level of P-glycoprotein expression, protein and RNA levels may be disproportionately higher or lower than expected for the level of resistance observed. This phenotypic diversity may be the result of both MDR1 mutations and of posttranslational modifications of the MDR1 gene product. Mutations in the coding region of the *MDR1* gene have been reported to alter the relative resistance patterns of cells. [58] P-glycoprotein can be phosphorylated by protein kinase C [59, 60] and by a novel membrane associated protein kinase. [61] Transport studies on MDR cells treated with protein kinase C activators and inhibitors, as well as with inhibitors of protein phosphatases, show that increased phosphorylation of P-glycoprotein is associated with decreased vinblastine accumulation. [60, 62, 63] Other cofactors involved in the augmentation of P-glycoprotein function have been proposed but not yet identified. [51, 64]

A thorough understanding of the regulation of P-glycoprotein production and the means to suppress its expression might significantly influence future cancer treatment strategies. Studies addressing this issue have shown that high levels of P-glycoprotein expression in vitro are often associated with *MDR* gene amplification and transcriptional activation. [8, 9] Increased expression of P-glycoprotein can also be stimulated by heat shock, [65] heavy metals, cytotoxic drugs,[66–68] regenerating liver, [66, 67] differentiating agents,[69–71] and by repeated exposure to ionizing radiation. [72] However, the responses to these treatments appear to vary between species and are cell line specific. Thus, predictable modulation of *MDR* gene expression is not yet possible. Under certain conditions in some cells, the *MDR1* promoter activity can be regulated by altered expression of oncogenes (*raf* and *ras*) and the tumor-suppressor gene, *p53*.[73–77]

A considerable literature has accumulated concerning the importance of P-glycoprotein in human cancer. P-glycoprotein RNA or protein has been detected in tumor specimens derived from patients with acute and chronic leukemias,[78–80] ovarian cancer, [81] multiple myeloma, [82] breast cancer, [83, 84] neuroblastoma, [85] soft tissue sarcomas, [86] renal cell carcinoma, [87] and others. [88] Although the numbers of patients with particular tumors in these studies were small, the results have tended to link increased P-glycoprotein expression with a history of prior therapy (usually with MDR-associated drugs) or toxin exposure, and poorer treatment outcome. In general, the relationship between increased P-glycoprotein and adverse outcome in human cancers is strongest in hematologic malignancies. Recently, three prospective studies have shown that increased P-glycoprotein in patients with acute myelogenous leukemia (AML) is associated with decreased complete remission rates and reduced remission duration with use of conventional chemotherapy.[89–91] This correlation has also been demonstrated in adult multiple myeloma, lymphoma, and pediatric ALL.[92–94] Moreover, efforts to

reverse clinical resistance to chemotherapy using P-glycoprotein inhibitors (see below) have similarly been most promising in the treatment of selected hematologic malignancies. [93,95–97]

Among solid tumors, the relationship between P-glycoprotein expresssion and response to therapy is less convincing, [98] although significant correlations between P-glycoprotein and adverse outcome in pediatric rhabdomyosarcoma and neuroblastoma have been reported. [92] Although P-glycoprotein was frequently present in tumor specimens from both treated and untreated patients with neuroblastoma, P-glycoprotein RNA tended to be higher in patients treated with regimens that included doxorubicin than in untreated patients. [85] Moreover, in patients with advanced neuroblastoma, P-glycoprotein expression has been strongly associated with aggressive biologic behavior, poor treatment response, and poor outcome. [99] The impressive correlations between P-glycoprotein expression and aggressive neuroblastoma persisted even when the data were corrected, by multivariant analyses, for other confounding prognostic features. However, the significance of *mdr1* expression in neuroblastomas is controversial as other data have suggested the opposite—that increased *mdr1* expression is associated with more favorable clinical variables in patients with neuroblastoma. [100] In tumor specimens obtained from patients with soft-tissue sarcomas, [86] the presence of P-glycoprotein was associated with anthracycline pretreatment, increased rate of remission induction failure, and increased frequency of relapse. In the more than 400 tumor specimens that were tested for P-glycoprotein RNA levels in a large study, [88] increased levels of *MDR1* RNA were more prevalent in tumors that tended to be intrinsically resistant to therapy (colon, renal, adrenal, hepatic, and pancreatic cancers) as compared with intrinsically sensitive tumors. Furthermore, P-glycoprotein RNA was often increased in tumors at relapse (acute leukemias, breast cancer, neuroblastoma, pheochromocytoma, and nodular poorly differentiated lymphoma).

Consequently, the available evidence indicates that P-glycoprotein overexpression is associated with clinical evidence of drug resistance and treatment failure in a significant number of patients—especially, selected groups with hematologic malignancies. However, additional and prospective studies are required to fully evaluate the clinical significance of P-glycoprotein in human cancer. These studies should include standardized P-glycoprotein RNA, protein and functional determinations in clinical specimens, and clearly defined clinical outcomes. [101] Such studies will help establish cancers for which the determination of P-glycoprotein levels in patients at diagnosis or relapse may have an important role in the design of treatment protocols. ↑ TOP

Multidrug Resistance Protein Family

Similar phenotypes of multiple resistance to antineoplastic agents have been described that are associated with the expression of other membrane proteins.

In many of these examples, resistance occurs independently of P-glycoprotein expression.[102–106] A distinct gene, *mrp1* (multidrug resistance protein 1 or multidrug resistance-associated protein 1), was isolated from a doxorubicin-selected MDR lung cancer cell line. [107] Except for the absence of P-glycoprotein expression, the phenotype of this cell line, which includes the property of reduced drug accumulation, was similar to classic MDR. The *mrp1* gene encodes a 190-kilodalton (kDa) transmembrane protein, whose structure is strikingly homologous to P-glycoprotein/MDR1 and other members of the ATP-binding cassette (ABC) transmembrane transporter proteins. [107, 108] Primary sequence analysis predicts the transmembrane structure shown in Figure 48-1. The structure, supported by immunochemical data, includes 11 plus 4 (or, alternatively, 11 plus 6) transmembrane domains with 2 cytosolic ATP-binding sites. [109] Increased MRP1 expression is associated with MDR, and decreased MRP1 expression is associated with reversion to drug sensitivity. Gene transfer experiments establish that MRP1 can confer MDR to a variety of drugs, including anthracyclines, epipodophyllotoxins, and vinca alkaloids.[110–112] Transport studies indicate that MRP1 is involved in ATP-dependent efflux of some native natural product anticancer drugs. Additionally, MRP1 is an ATP-dependent transporter of a variety of anionic conjugates of drugs and other xenobiotics—conjugates that include glutathione conjugates, glucuronides, and sulfates.[113–118] Thus, MRP1 is an important xenobiotic-conjugate transport pump that is involved in efflux detoxification of a wide range of cellular poisons, including anticancer drugs and their conjugates. The significance of these conjugate substrates is further discussed in a following section. In contrast to P-glycoprotein, whose substrates are generally lipophilic neutral or cationic compounds, MRP1 preferentially recognizes amphiphilic organic anions including the conjugates described above. While neutral, hydrophobic compounds such as vincristine are also substrates of MRP1, reduced glutathione is required for their transport. [116, 119] Although no covalent linkage between glutathione and vincristine is observed, it is believed that both glutathione and the neutral drug must be simultaneously present to effect efflux, and that they both may be cotransported by MRP1.

MRP1 is ubiquitously expressed in tumor and normal tissues.[120–122] The importance of MRP1 overexpression in clinical drug resistance is unknown. However, because levels of MRP1 expression vary widely in tumor cells, MRP1 may be a significant mediator of drug resistance in human cancer.

There are at least five other human MRP isoforms identified. [35, 123] Among them, MRP2 (cMOAT) and MRP3 are also capable of supporting efflux detoxification of cancer drugs, including epipodophyllotoxins (MRP2 and 3), doxorubicin, and cisplatin (MRP2). [124, 125] Recent results indicate that MRP1, MRP2, MRP3 and MRP4 can all act as methotrexate efflux pumps and can confer resistance to methotrexate. [125, 126] Unlike MRP1, which is expressed on the basolateral plasma membrane surface of polarized cells, MRP2 is normally targeted to the apical membrane surface of bile canalicular and renal tubular epithelium.[127–129] MRP3 is localized to the basolateral

surface in various tissues, including the colon, liver, and pancreas, [35, 130, 131] and can also confer low-level resistance to etoposide and teniposide. [125] TOP

MDR Associated with Topoisomerase Poisons

Topoisomerases are nuclear enzymes that catalyze the formation of transient single- or double-stranded DNA breaks, facilitate the passage of DNA strands through these breaks, and promote rejoining of the DNA stands. [132, 133] As a consequence of these activities, topoisomerases are thought to be critical for DNA replication, transcription, and recombination. The cytotoxicity of many drugs that target topoisomerases, a class of drugs here termed *topoisomerase poisons* (Table 48-4), is thought to depend on the DNA cleavage activities of topoisomerases. There are two classes of mammalian enzymes, topoisomerases I and II. Topoisomerase I catalyzes the formation of single-stranded DNA breaks, while topoisomerases II (α and β isoforms) catalyze both single- and double-stranded breaks. During the cleavage reactions reversible DNA-topoisomerase complexes (cleavable complexes) can be stabilized by interactions with topoisomerase poisons. The formation of these stabilized DNA-topoisomerase-drug complexes is thought to initiate the production of lethal DNA strand breaks. Of the chemotherapeutic drugs that affect topoisomerase activities, the topoisomerase II poisons have been the most important clinically. A partial list of these agents, which include DNA intercalating and nonintercalating drugs, appears in Table 48-3. A growing list of useful topoisomerase I poisons are now available, including topotecan, CPT-11 (irinotecan), and SN-38.

Several laboratories have described an MDR pattern characterized by resistance of cells to several or all of the drugs listed in Table 48-3.[134, 135] It is readily apparent that many of these topoisomerase II-targeting drugs are also members of the classic MDR phenotype (see Table 48-2). Hence, decreased drug accumulation via increased expression of P-glycoprotein or MRP1 represents a potential mechanism of resistance to these topoisomerase II poisons. However, a distinct pattern of the topoisomerase II-related MDR has been described that differs from the pattern of P-glycoprotein-associated MDR in several important ways. First, resistance to these drugs is not usually associated with reduced drug accumulation or P-glycoprotein expression. Exceptions may reflect the presence of multiple simultaneous mechanisms of resistance. Additionally, cells that display this topoisomerase II-related resistance phenotype are usually sensitive to antimicrotubule drugs associated with classic MDR, including Vinca alkaloids and colchicine, unless a concomitant drug transport or microtubule alteration exists. The mechanism of resistance to topoisomerase II poisons is thought to involve altered topoisomerase II activity. Both qualitative and quantitative changes in enzyme activity have been demonstrated in resistant cell lines. Reduced levels of topoisomerase activity are associated with decreased drug-induced DNA strand breaks, as well as reduced drug cytotoxicity. [136, 137] Other studies implicate intrinsic changes in drug-induced catalytic properties or

associated cofactors as the basis of drug resistance in some cells. [28,138–140] The nature of the topoisomerase II alterations may influence the cross-resistance patterns observed. For example, cells that develop alterations in topoisomerase II following exposure to m-AMSA (amsacrine) may show cross-resistance to other intercalating topoisomerase II poisons, but not to epipodophyllotoxins. [139] Collectively, these data indicate that reduced topoisomerase protein levels or selectively altered enzyme activities influencing drug-enzyme interactions may render cells relatively more resistant to drugs by interfering with the formation of stable cleavable complexes and hence cytotoxic DNA strand breaks. Indeed, the normal downregulation of topoisomerase II in nondividing cells [132] may explain the relative insensitivity to topoisomerase II poisons of some solid tumors containing a large proportion of quiescent cells. Finally, there are two mammalian isozymes of topoisomerase II, a 170-kDa form (topoisomerase IIα) and a 180-kDa form (topoisomerase IIβ).[141–143] These isozymes differ with respect to their regulation during the cell cycle [144] and their relative sensitivities to topoisomerase II poisons. [141, 142] Hence, the relative levels of the specific topoisomerase II isozymes as well as the total topoisomerase II activity may be significant determinants of the sensitivity of tumor cells to topoisomerase II drugs.

Several reports suggest the molecular bases of drug resistance associated with qualitatively altered topoisomerase II. [145] Point mutations leading to amino acid substitutions in topoisomerase IIα isolated from cells selected for resistance to topoisomerase II drugs have been described. These mutations are clustered within the conserved ATP-binding consensus sequences [31,146–149] or near the Tyr 804 residue involved in covalent attachment of topoisomerase IIα to DNA.[148–150] Although these topoisomerase IIα mutations are associated with drug resistance in intact cells and, in some cases, with altered enzymatic activities in vitro, the exact mechanism(s) of drug resistance and the relationship of these mutations to a specifically altered enzymatic property are incompletely understood. Moreover, the relevance for clinical drug resistance of these topoisomerase IIα mutations identified in experimentally drug-selected resistant cell lines is unknown. Indeed, one study of topoisomerase IIα derived from leukemic blasts of 15 relapsed patients failed to identify mutations in either of the above two regions implicated in experimental drug resistance. [148] Other qualitative alterations in topoisomerase II activity and structure have been described in cell lines selected for resistance to topoisomerase II poisons. These alterations include a selective decrease in nuclear matrix-associated topoisomerase II [151] and a truncated form of topoisomerase IIα . [29] In some resistant cell lines, cytoplasmic or membrane components may be responsible for the altered topoisomerase II activity implicated in the emergence of drug resistance. [152] Alternatively, altered subcellular localization of topoisomerase II isoforms [145, 153, 154] or altered posttranslational phosphorylation [145, 155] have been reported in association with some etoposide-resistant cell lines.

The cytotoxicity of topoisomerase II poisons is believed to depend on the formation of DNA strand breaks secondary to stabilization of the reversible enzyme-DNA cleavable complex. [132] It is thought that a collision between the complex and the DNA replication fork is necessary to generate the cytotoxic lesions. If DNA replication is delayed or altered until after the drug is cleared, the cleavable complex can be reversed and the cytotoxic lesion does not form. [145] Thus, altered DNA replication or repair timing could also mediate topoisomerase II poison resistance.

A new family of drugs targeting topoisomerase II function has emerged that includes fostriecin, merbarone, aclarubicin, and *bis* (2,6-dioxopiperazine) derivatives (eg, ICRF 193 and ICRF 187). In contrast to the topoisomerase II poisons that stabilize cleavable complexes (see above and Table 48-4), this new family of drugs target the catalytic cycle of topoisomerase II activity in which DNA strands are intact. Because the toxicity of these "catalytic inhibitors" is independent of cleavable complex stablization, cross-resistance with the topoisomerse II poisons is less likely. [145, 156, 157]

The cytotoxic agent camptothecin enhances topoisomerase I-mediated strand breaks. Earlier, host toxicity prohibited the clinical use of such topoisomerase I poisons. However, the prospect of less-toxic analogs of this drug that maintain a high level of activity against topoisomerase I-rich human cancer cells has renewed interest in the clinical application of this class of compounds. [158] Consequently, the emergence of resistance to these agents may become an increasingly important consideration. There are reports of topoisomerase I mutations derived from cell lines selected for resistance to camptothecin or its derivative, CPT-11. [30, 159, 160] In two of these resistant cell lines, the mutant enzyme has altered topoisomerase I activity with a reduced capacity to mediate camptothecin-induced DNA strand breaks.[159–161] TOP

MDR Associated with Altered Expression of Drug-Metabolizing Enzymes and Drug-Conjugate Export Pumps

The manner in which cells metabolize cancer drugs and other xenobiotics is often described as three phases of detoxifications (Figure 48-2). [162] While none of these phases are obligatory steps in the metabolism of every drug, the concept illustrated in Figure 48-2 represents a useful framework with which to view cellular detoxification mechanisms. Alterations in any of these three phases can influence the sensitivity or resistance to a particular drug or xenobiotic toxin. Phase I metabolism is mediated by cytochome P450 mixed-function oxidases. Generally, the drug or xenobiotic is rendered a more electrophilic, reactive intermediate—a process that may enhance toxicity. These metabolites, or the unmodified drug, may then be converted to a less-reactive, presumably less-toxic, form in phase II reactions. Phase II detoxifications include the formation of drug/xenobiotic conjugations with glutathione (GSH), glucuronic acid, or sulfate—reactions that are catalyzed by multiple isozymes each of glutathione *S*-transferase (GST), uridine

diphosphate (UDP)-glucuronosyl transferase, and sulfatase, respectively.[163-167] Phase III detoxification consists of export of the parent drug/xenobiotic or its metabolites by energy-dependent transmembrane efflux pumps, including MRP family members as described above.

Frequently, in cellular and animal models of drug or xenobiotic resistance, a coordinated downregulation of phase I drugactivating enzymes and an upregulation of specific phase II drug-conjugating enzymes is observed. [66, 67,168-169] Such a programmed cellular stress response offers a versatile, generalized protective mechanism against exposure to a variety of exogenous toxins.

Of the phase II enzymes, the GSTs have been the most extensively studied. GSTs[163, 164] comprise multiple soluble and membraneassociated isozymes, which catalyze the conjugation of electrophilic, hydrophobic compounds (R-X) with the thiol, GSH:

$$R-X+GSH \xrightarrow{GST} R-SG+HX$$

Circumstantial evidence links the increase in specific GST isozymes or bulk GST activity in cells to resistance to alkylating agents and other drugs.[163, 164,170-173] However, direct evidence that GSTs are responsible for altering drug sensitivities is limited. Another catalytic activity, selenium-independent glutathione peroxidase activity, has been attributed to some isozymes of GST:

$$R-O-OH+2GSH \xrightarrow{GST} R-OH+GSSG+H_2O$$

This and other GST-mediated reactions are of interest because of their potential to detoxify oxidative damage to membranes and DNA.

Studies using cell-free preparations of GSTs have identified a limited number of antineoplastic drug substrates of these enzymes. Table 48-5 lists these drugs and other substrates that are possibly associated with drug mediated-oxidative damage. Whether GST levels in tumor cells are sufficient to detoxify antineoplastic drugs to a clinically significant extent is a matter of considerable debate. Several cancer drugs, particularly reactive electrophilic alkylating agents, can form conjugates with glutathione—both spontaneously and in enzyme-catalyzed reactions.[174-181] However, despite these catalytic activities, the role of GSTs in drug resistance remains uncertain because of inconsistent results from different laboratories. [172,181-191] Indeed, some investigators have found an association between cellular resistance to some anticancer drugs and expression of a particular isozyme of GST, whereas others have found no such association.

The importance of drug/xenobiotic-conjugate transporters for cellular export and detoxification of certain compounds is increasingly appreciated. Conjugation frequently renders the parent drug more hydrophilc and less capable of diffusing the plasma membrane—trapping the drug within the cell. While conjugation with glutathionyl or glucuronosyl groups may render some drugs less toxic, these drug conjugates themselves may retain significant toxicity. For example, the glutathione conjugate formed with cisplatinum is itself toxic and an inhibitor of protein synthesis. [192] Moreover, drug conjugates may inhibit their conjugating enzyme(s). [193] Thus, the relative resistance of cells expressing drug-metabolizing enzymes may depend on cellular levels of drug conjugate transporters, including the glutathione conjugate transporters, [162, 194] such as the MRP family proteins. [116, 117] Indeed, recent results using model cell lines demonstrate that combined expression of specific isozymes of GST with MRP1 is necessary to achieve full protection from the toxicities of the cancer drug chlorambucil, [41] or from the carcinogen 4-nitroquinoline 1-oxide. [195] In these studies, the expression of either GST or MRP1 alone provided little, if any, protection from toxicity—a finding that illustrates the synergistic interaction of phase II and phase III detoxification processes in the emergence of resistance to some drugs and other xenobiotics. TOP

Emergence of Refractory Tumors Associated with Multiple Resistance Mechanisms

The backbone of many treatment protocols designed to circumvent the proliferation of resistant tumor cells is the administration of multiple drugs with different structural properties and mechanisms of action. The approach supposes that if enough carefully selected drugs are delivered at optimal doses and intervals, individual clones of cells resistant to one class of drug will be effectively killed by another drug in the regimen. The rapid appearance of refractory tumors despite an initially favorable cytoreductive response suggests that the emergence of tumor cell clones with multiple resistance is a common clinical occurrence. We have seen how a single genetic change, such as increased P-glycoprotein or altered topoisomerase II, can mediate cross-resistance to several, but not all, useful antineoplastic drugs. Although these mechanisms provide a molecular explanation for broad-spectrum resistance, it is clear that many refractory tumor clones must simultaneously develop multiple resistance mechanisms. These mechanisms may arise from multiple independent genetic changes in single-cell clones or, as suggested by Cadman, from cell-to-cell transfer of genetic information.[196] TOP

Resistance to Anticancer Genotoxic Treatments Related to Suppression of Apoptotic Pathways

Chemotherapeutic drugs initiate cytotoxicity through their interactions with a variety of molecular targets. For example, epipodophyllotoxins attack topoisomerases II, alkylating agents form adducts with the nucleophilic

centers of DNA and proteins, and methotrexate inhibits dihydrofolate reductase, resulting in reduced pyrimidine and purine synthesis. Despite these varied primary targets, most, if not all, cancer drugs effect cell death, at least partially, via downstream events—events that converge upon pathways mediating programmed cell death or apoptosis.

Apoptosis refers to an orderly cellular death program with predictable molecular and morphologic changes, including nuclear pyknosis and fragmentation, internucleosomal endonucleolytic DNA fragmentation, formation of cytoplasmic apoptotic bodies, and plasma membrane changes, such as transposition of phosphatidylserine to the extracellular surface. [197] The process is conveniently conceptualized in three phases. First, initiation of apoptosis (eg, secondary to chemotherapy-mediated DNA damage) is characterized by its reversibility. Second, commitment represents the irreversible decision to complete the death program. The commitment phase may involve mitochondrial changes including the permeability phase transition and the release of cytochrome c and apoptosis-inducing factor (AIF)-changes that are hallmarks of apoptosis. Finally, the degradation or execution phase includes downstream events, including DNA fragmentation and morphologic changes. Prior to commitment, apoptosis can be modulated by regulatory elements, such as p53 and the Bcl-2 family proteins. [20,197–199] Clearly, such regulation of the apoptotic response can have profound effects on the outcome of chemotherapy and is an area of active investigation germane to drug resistance and sensitivity.

Although apoptosis may be either p53-dependent or independent, frequently the cellular response to DNA damage is regulated by p53. [199] As shown in a simplified diagram (Figure 48-3), cancer therapy-induced DNA damage is sensed by p53 by incompletely understood mechanisms. Depending on the particular cell type and damage, p53 may then initiate one of two possible pathways: apoptosis or a process of cell-cycle arrest and repair. In cells where the apoptotic pathway dominates, changes that cause dysfunction or deletion of p53 are likely to result in reduced apoptosis in response to DNA damage, leading to relative resistance and cell survival with damage. Indeed, p53 is required for radiation- and etoposide-induced apoptosis in thymocytes, whereas lymphoma cell lines expressing mutant p53 were relatively resistant to DNA-damaging agents. [200–202] In cells where the p53-dependent cell-cycle arrest and repair response dominates, deletion or mutation of p53 might be expected to result in decreased cell-cycle arrest and repair leading to accumulated DNA damage and hence sensitivity to the chemotherapeutic agent. [199]

The mitogen-activated protein kinase (MAPK)-signaling cascades are involved in the regulation of cellular response to exogenous factors, including geno- and cytotoxic cancer treatments. [203] The extracellular stimulus-regulated kinase (ERK1/2) pathway is implicated in the proliferative response to growth factors. In cells treated with potentially cytotoxic stressors, such as radiation or anticancer drugs, the p38 and stress-activated/c-Jun *N*-terminal protein kinase (SAPK/JNK) pathways are

implicated in mediating cell-cycle arrest or apoptosis. Modulation of these interacting pathways can have a profound effect on whether a cancer cell responds to cytotoxin challenge by activation of apoptosis or by cell-cycle arrest, repair, and hence relative resistance to treatment. [203, 204]

The Bcl-2 family proteins comprise several important regulators of apoptosis. Although their mechanism(s) of action is incompletely known, the balance of expressed antiapoptotic family members (Bcl-2, Bcl-X_L, Bcl-w, A1, and Mcl-1) and proapoptotic family members (Bax, Bak, Bad, Bik, and Bid) can influence the relative sensitivity of cells to toxic stressors. [20, 198] Indeed, increased Bcl-2 and its antiapoptotic homologs are associated with increased resistance of lymphoid cells to the cytotoxic effects of corticosteroids, radiation, and DNA damage from chemotherapeutic drugs. [198,205–209] It has been proposed that increased levels of antiapoptotic proteins Bcl-2 or Bcl-X_L may result in reduced sensitivity to DNA-damaging cancer drugs—a resistance phenotype characterized by cell survival with increased tolerance of DNA damage and genomic instability. This genomic instability may further lead to mutations activating additional resistance mechanisms and conferring more aggressive tumor behavior. [20] Thus, the expression of mutant and wild-type p53, Bcl-2 family members, MAPK family members, and other proteins associated with the control of apoptosis may contribute significantly to the clinical sensitivity of tumor cells. These proteins are the targets of investigational agents that may become important in future strategies to overcome clinical drug resistance. ↑ TOP

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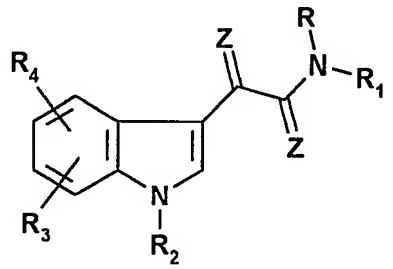
Die folgenden Angaben sind den vom Anmelder eingereichten Unterlagen entnommen

Prüfungsantrag gem. § 44 PatG ist gestellt

(54) Indolyl-3-glyoxylsäure-Derivate mit Antitumorwirkung

(57) Die Erfindung betrifft die Verwendung von N-substituierten Indol-3-gloxylamiden der allgemeinen Formel 1 als Antitumormittel



Formel 1

sowie pharmazeutische Zusammensetzung mit Antitumorwirkung, gekennzeichnet durch einen Gehalt an mindestens einer der Verbindungen der allgemeinen Formel 1 ggf. auch in Form der physiologisch verträglichen Säureadditionssalze oder N-Oxide. Ferner umfaßt die Erfindung auch Antitumormittel, enthaltend als aktiven Wirkstoff ein oder mehrere N-substituierte Indol-3-gloxylamide gemäß der allgemeinen Formel 1 sowie ggf. deren physiologisch verträglichen Säureadditionssalze und, sofern möglich, N-Oxide und einen pharmazeutisch verwendbaren Träger- und/oder Verdünnungs- bzw. Hilfsstoffen zur Infusion oder Ampullen, Suppositorien, Pflaster, inhalativ einsetzbaren Pulverzubereitungen, Suspensionen, Cremes und Salben.

DE 198 14 838 A 1

Beschreibung

Indol-3-glyoxylamide finden als pharmakodynamisch aktive Verbindungen und als Synthesebausteine in der pharmazeutischen Chemie eine vielfältige Verwendung.

In der Patentanmeldung Neth. Appl. 6502481 sind Verbindungen beschrieben, die über ein antiinflammatorisches und antipyretisches Wirkprofil und analgetische Aktivität verfügen.

In der britischen Anmeldung GB-PS 1 028 812 finden Derivate der Indolyl-3-glyoxylsäure und deren Amide Erwähnung als analgetisch, antikonsulsiv und β-adrenergisch wirksame Verbindungen.

G. Domschke et al. (Ber. 94, 2353 (1961)) beschreibt 3-Indolyl-glyoxylamide, die pharmakologisch nicht charakterisiert sind.

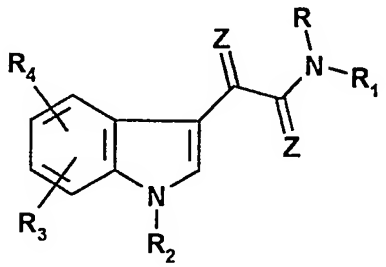
E. Walton berichtet in J.Med.Chem. 11, 1252 (1968) über Indolyl-3-glyoxylsäure-Derivate, die inhibitorisch auf die Glycerophosphat-Dehydrogenase und Lactat-Dehydrogenase wirken.

In der Europäischen Patentschrift EP 675110 werden 1H-Indol-3-glyoxylsäureamide beschrieben, die als sPLA2-Inhibitoren profiliert werden und bei Behandlung des septischen Schocks, bei Pankreatitis, bei der Behandlung allergischer Rhinitis und rheumatischer Arthritis zur Anwendung kommen.

Ziel der vorliegenden Erfindung ist es, N-substituierte Indol-3-glyoxylamide zur Verfügung zu stellen, die eine Antitumor-Wirkung besitzen und somit den verfügbaren Arzneischatz zu bereichern.

Die genannten Verbindungen sind bereits als Arzneimittel mit antiasthmatischer, antiallergischer und immunsuppressiver/immunmodulierender Wirkung aus DE-OS 196 36 150 A1 bekannt.

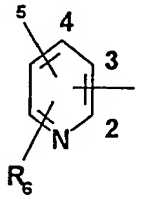
Der Gegenstand der Erfindung umfaßt daher die Verwendung von N-substituierten Indol-3-gloxylamiden der allgemeinen Formel 1 zur Herstellung von Antitumormitteln, Antitumormittel mit einem Gehalt an Wirksubstanz gemäß Formel 1 und deren Einsatz zur Behandlung von Tumorerkrankungen.



Formel 1

wobei die Reste R, R_1, R_2, R_3, R_4 und Z folgende Bedeutung haben:
R = Wasserstoff, (C_1-C_6)-Alkyl, wobei die Alkylgruppe ein- oder mehrfach durch den Phenylring substituiert sein kann und dieser Phenylring seinerseits ein- oder mehrfach durch Halogen, (C_1-C_6)-Alkyl, (C_3-C_7)-Cycloalkyl, durch Carboxylgruppen, mit C_1-C_6-Alkanolen veresterte Carboxylgruppen, Trifluormethylgruppen, Hydroxylgruppen, Methoxygruppen, Ethoxygruppen, Benzyloxygruppen sowie durch eine im Phenylteil ein- oder mehrfach mit (C_1-C_6)-Alkylgruppen, Halogenatomen oder Trifluormethylgruppen substituierte Benzylgruppe substituiert sein kann,
R steht ferner für die Benzyloxycarbonyl-Gruppe (Z-Gruppe) und für den tertiär-Butoxycarbonylrest (Boc-Rest), weiterhin für die Acetylgruppe.

R_1 kann den Phenylring, der ein- oder mehrfach mit (C_1-C_6)-Alkyl, (C_1-C_6)-Alkoxy, Cyano, Halogen, Trifluormethyl, Hydroxy, Benzyloxy, Nitro, Amino, (C_1-C_6)-Alkylamino, (C_1-C_6)-Alkoxycarbonylamino und mit der Carboxylgruppe bzw. mit der mit C_1-C_6-Alkanolen veresterten Carboxylgruppe substituiert ist, oder ein Pyridin-Gerüst der Formel 2 und deren N-Oxid



Formel 2

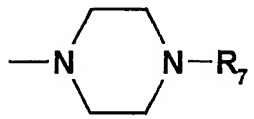
bedeuten und dessen N-Oxid, wobei das Pyridin-Gerüst wahlweise an den Ringkohlenstoff Atomen 2, 3 und 4 gebunden ist und mit den Substituenten R_5 und R_6 substituiert sein kann. Die Reste R_5 und R_6 können gleich oder verschieden sein und die Bedeutung (C_1-C_6)-Alkyl sowie die Bedeutung (C_3-C_7)-Cycloalkyl, (C_1-C_6)-Alkoxy, Nitro, Amino, Hydroxy, Halogen und Trifluormethyl besitzen und ferner den Ethoxycarbonylamino-Rest sowie die Gruppe Carboxyalkyloxy darstellen, bei dem die Alkylgruppe über 1–4 C-Atome verfügen kann.

R_1 kann ferner ein 2- bzw. 4-Pyrimidinyl-Heterocyclus sein, wobei der 2-Pyrimidinyl-Ring ein- oder mehrfach mit der Methylgruppe substituiert sein kann, weiterhin das mit (C_1-C_6)-Alkyl, Halogen, der Nitrogruppe, der Aminogruppe und dem (C_1-C_6)-Alkylamino-Rest substituierte 2-,3-, und 4- und 8-Chinolylgerüst bedeuten, eine 2-, 3- und 4-Chinolylmethylgruppe darstellen, wobei die Ringkohlenstoffe des Pyridylmethylrestes der Chinolylgruppe und des Chinolylmethyl-Restes mit (C_1-C_6)-Alkyl, (C_1-C_6)-Alkoxy, Nitro, Amino und (C_1-C_6)-Alkoxycarbonylamino substituiert sein können.

R_1 kann weiterhin für den Fall, daß R = Wasserstoff, die Methyl -oder Benzylgruppe sowie den Benzyloxycarbonyl-Rest (Z-Rest), den tert.-Butoxycarbonyl-Rest (BOC-Rest) und die Acetylgruppe darstellt, die folgenden Reste bedeuten: $-CH_2COOH$; $-CH(CH_3)-COOH$; $-(CH_3)_2-CH-(CH_2)_2-CH-COO-$; $H_3C-H_2C-CH(CH_3)-CH(COOH)-$; $HO-H_2C-CH(COOH)-$; Phenyl-CH_2-CH(COOH)-; (4-Imidazolyl)-CH_2-CH-(COOH)-; $HN=C(N\ H_2)-NH-(CH_2)_3-CH(COOH)-$; $H_2N-(CH_2)_4-CH(COOH)-$; $H_2N-CO-CH_2-CH-(COOH)-$; $HOOC-(CH_2)_2-CH(COOH)-$;

R_1 kann weiterhin für den Fall, daß R Wasserstoff, die Z-Gruppe, den BOC-Rest, die Acetyl- oder die Benzylgruppe bedeuten, der Säurerest einer natürlichen oder unnatürlichen Aminosäure sein, z. B. den α-Glycyl-, den α-Sarkosyl-, den α-Alanyl-, den α-Leucyl-, den α-iso-Leucyl-, den α-Seryl-, den α-Phenylalanyl-, den α-Histidyl-, den α-Prolyl-, den α-Arginyl-, den α-Lysyl-, den α-Asparagyl- und den α-Glutamyl-Rest darstellen, wobei die Aminogruppen der jeweiligen Aminosäuren ungeschützt vorliegen oder geschützt sein können. Als Schutzgruppe der Aminofunktion kommen der Carbobenzoxy-Rest (Z-Rest) und der tert.-Butoxycarbonyl-Rest (BOC-Rest) sowie die Acetylgruppe in Frage. Im Fall des für R_1 beanspruchten Asparagyl- und Glutamylrestes liegt die zweite, nicht gebundene Carboxylgruppe als freie Carboxylgruppe oder in Form eines Esters mit C_1-C_6-Alkanolen, z. B. als Methyl-, Ethyl- bzw. als tert.-Butylester vor.

Weiterhin kann R_1 die Allylaminocarbonyl-2-methyl-prop-1-yl-Gruppe bedeuten. R und R_1 können ferner zusammen mit dem Stickstoff-Atom, an das sie gebunden sind, einen Piperazinring der Formel 3 oder einen Homopiperazinring bilden, sofern R_1 eine Aminoalkylengruppe darstellt, bei dem



Formel 3

R_7 einen Alkylrest darstellt, einen Phenylring bedeutet, der ein- oder mehrfach mit (C_1-C_6)-Alkyl, (C_1-C_6)-Alkoxy, Halogen, der Nitrogruppe, der Aminofunktion und mit der (C_1-C_6)-Alkylaminogruppe substituiert sein kann. R_7 bedeutet ferner die Benzhydryl-Gruppe und die Bis-p-fluorbenzylhydryl-Gruppe.

R_2 kann Wasserstoff und die (C_1-C_6)-Alkyl-Gruppe bedeuten, wobei die Alkylgruppe ein- oder mehrfach durch Halogen und Phenyl substituiert ist, das seinerseits ein- oder mehrfach durch Halogen, (C_1-C_6)-Alkyl, (C_3-C_7)-Cycloalkyl, Carboxylgruppen mit C_1-C_6-Alkanolen veresterten Carboxylgruppen, Trifluormethylgruppen, Hydroxylgruppen, Methoxygruppen, Ethoxygruppen oder Benzyloxygruppen substituiert sein kann. Die für R_2 geltende (C_1-C_6)-Alkyl-Gruppe kann ferner durch die 2-Chinolylgruppe und das 2-,3- und 4-Pyridyl-Gerüst substituiert sein, die beide jeweils ein- oder mehrfach durch Halogen, (C_1-C_4)-Alkylgruppen oder (C_1-C_4)-Alkoxy-gruppen substituiert sein können. R_2 steht ferner für den Aroyl-Rest, wobei der diesem Rest zugrunde liegende Arylteil den Phenylring darstellt, der ein- oder mehrfach durch Halogen, (C_1-C_6)-Alkyl, (C_3-C_7)-Cycloalkyl, Carboxylgruppen mit C_1-C_6-Alkanolen veresterte Carboxylgruppen, Trifluormethylgruppen, Hydroxylgruppen, Methoxygruppen, Ethoxygruppen oder Benzyloxygruppen substituiert sein kann.

R_3 und R_4 können gleich oder verschieden sein und Wasserstoff, (C_1-C_6)-Alkyl, (C_3-C_7)-Cycloalkyl, (C_1-C_6)-Alkanoyl, (C_1-C_6)-Alkoxy, Halogen und Benzyloxy bedeuten.

Weiterhin können R_3 und R_4 die Nitrogruppe, die Aminogruppe, die (C_1-C_4)-mono- oder dialkylsubstituierte Aminogruppe, und die (C_1-C_6)-Alkoxy-carbonylamino-Funktion oder (C_1-C_6)-Alkoxycarbonylamino-(C_1-C_6)-alkyl-Funktion bedeuten.

Z steht für O und S.

Unter der Bezeichnung Alkyl-, Alkanol-, Alkoxy- oder Alkylaminogruppe sind für die Reste R, R_1, R_2, R_3, R_4, R_5, R_6, R_7 regelmäßig sowohl "geradkettige" als auch "verzweigte" Alkylgruppen zu verstehen, wobei "geradkettige Alkylgruppen" beispielsweise Reste wie Methyl, Ethyl, n-Propyl, n-Butyl, n-Pentyl, n-Hexyl bedeuten können und "verzweigte Alkylgruppen" beispielsweise Reste wie Isopropyl oder tert.-Butyl bezeichnen. Unter "Cycloalkyl" sind Reste wie beispielsweise Cyclopropyl, Cyclobutyl, Cyclopentyl, Cyclohexyl oder Cycloheptyl zu verstehen.

Die Bezeichnung "Halogen" steht für Fluor, Chlor, Brom oder Jod. Die Bezeichnung "Alkoxygruppe" stellt Reste wie beispielsweise Methoxy, Ethoxy, Propoxy, Butoxy, Isopropoxy, Isobutoxy oder Pentoxy dar.

Die Verbindungen können auch als Säureadditionssalze eingesetzt werden, beispielsweise als Salze von Mineralsäuren, wie beispielsweise Salzsäure, Schwefelsäure Phosphorsäure, Salze von organischen Säuren, wie beispielsweise Essigsäure, Milchsäure, Malonsäure, Maleinsäure, Fumarsäure, Gluconsäure, Glucuronsäure, Zitronensäure, Embonsäure, Methansulfonsäure, Trifluoressigsäure, Bernsteinsäure und 2-Hydroxyethansulfonsäure.

Sowohl die Verbindungen der Formel 1 als auch deren Salze sind biologisch aktiv. Die Verbindungen der Formel 1 können in freier Form oder als Salze mit physiologisch verträglichen Säuren verabreicht werden.

Die Applikation kann peroral, parenteral, intravenös, transdermal oder inhalativ vorgenommen werden.

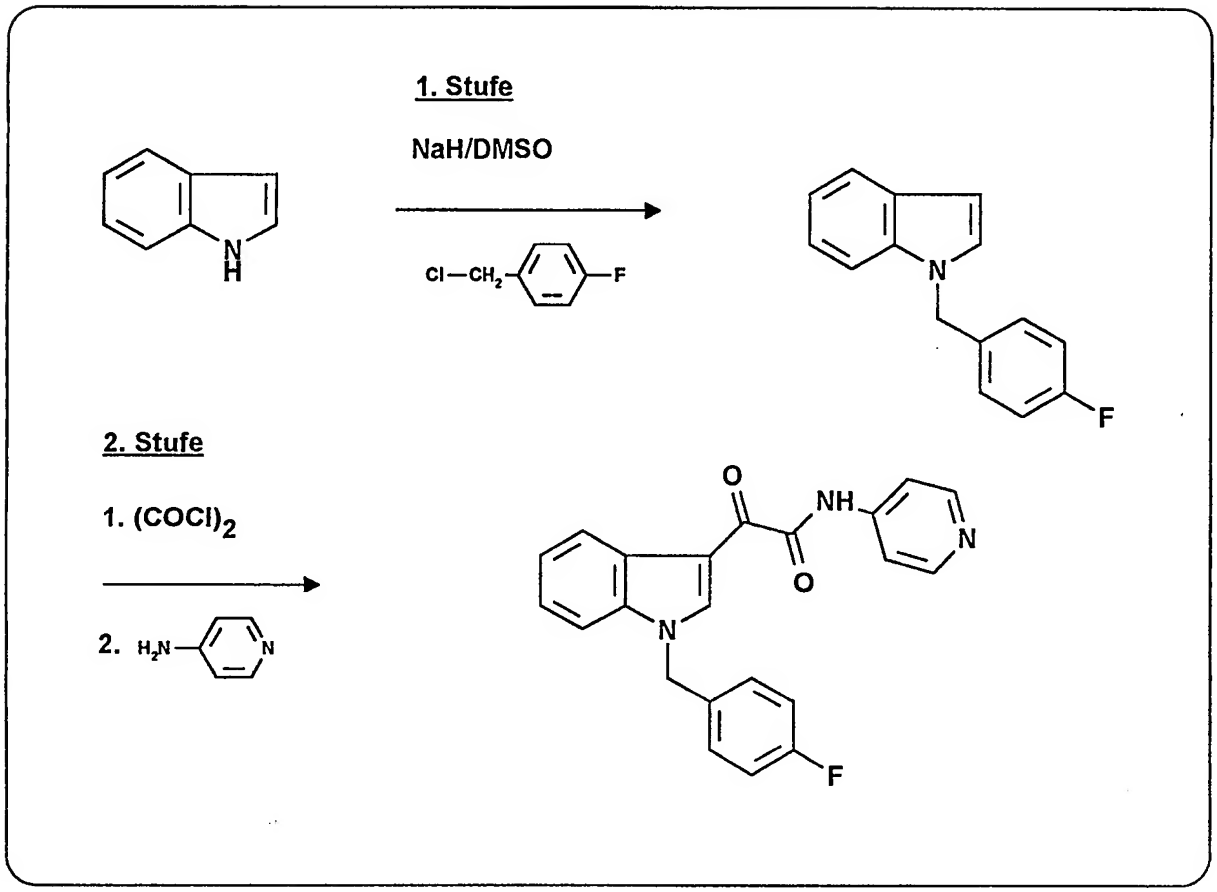
Weiterhin betrifft die Erfindung pharmazeutische Zubereitungen mit einem Gehalt an mindestens einer der Verbindungen der Formel 1 oder deren Salze mit physiologisch verträglichen anorganischen oder organischen Säuren und gegebenenfalls pharmazeutisch verwendbaren Träger- und/oder Verdünnungs- bzw. Hilfsstoffen.

Als Applikationsformen eignen sich beispielsweise Tabletten, Dragees, Kapseln, Lösungen zur Infusion oder Ampullen, Suppositorien, Pflaster, inhalativ einsetzbare Pulverzubereitungen, Suspensionen, Cremes und Salben.

Die Verfahren zur Herstellung der erfindungsgemäßen Verbindungen werden in den folgenden Reaktionsschemata 1 und 2 sowie in allgemeinen Vorschriften beschrieben. Alle Verbindungen lassen sich, wie beschrieben, oder analog herstellen.

Die Verbindungen der allgemeinen Formel 1 mit Z = O, R_1 = Aryl, Aralkyl, Heteroaryl und Heteroaralkyl sowie R_2 = Alkyl, Aralkyl und Heteroaralkyl sind gemäß des folgenden Schemas 1 erhältlich:

Schema 1



1. Stufe

Das Indol-Derivat, das unsubstituiert oder an C-2 oder im Phenylgerüst einfach oder mehrfach substituiert sein kann, wird in einem protischen, dipolar aprotischen oder unpolaren organischen Lösungsmittel, wie beispielsweise Isopropanol, Tetrahydrofuran, Dimethylsulfoxid, Dimethylformamid, Dimethylacetamid, N-Methylpyrrolidon, Dioxan, Toluol oder Methylenchlorid gelöst und tropfenweise zu einer in einem Dreihalskolben unter N_2-Atmosphäre vorbereiteten molaren oder überschüssig eingesetzten Suspension einer Base, wie beispielsweise Natriumhydrid, pulversiertes Kaliumhydroxid, Kalium-tert.-butylat, Dimethylaminopyridin oder Natriumamid in einem geeigneten Lösungsmittel gegeben. Sodann gibt man beispielsweise das gewünschte Alkyl-, Aralkyl- bzw. Heteroaralkylhalogenid gegebenenfalls unter Zusatz eines Katalysators, wie z. B. Kupfer, zu und läßt einige Zeit, beispielsweise 30 Minuten bis 12 Stunden, nachreagieren und hält die Temperatur innerhalb eines Bereichs von 0°C bis 120°C, vorzugsweise zwischen 30°C bis 80°C, besonders zwischen 50°C und 65°C. Nach Beendigung der Reaktion wird die Reaktionsmischung in Wasser gegeben, die Lösung z. B. mit Diethylether, Dichlormethan, Chloroform, Methyl-tert.-butylether oder Tetrahydrofuran extrahiert und die jeweils erhaltene organische Phase mit wasserfreiem Natriumsulfat getrocknet. Man engt die organische Phase im Vakuum ein, kristallisiert den verbleibenden Rückstand durch Anreiben oder reinigt den öligen Rückstand durch Umkristallisation, Destillation oder durch Säulen- bzw. Flash-Chromatographie an Kieselgel oder Aluminiumoxid. Als Laufmittel dient beispielsweise ein Gemisch aus Dichlormethan und Diethylether im Verhältnis 8 : 2 (Vol/Vol) oder ein Gemisch aus Dichlormethan und Ethanol im Verhältnis 9 : 1 (Vol/Vol).

2. Stufe

Das nach obenstehender Vorschrift der 1. Stufe erhaltene N-substituierte Indol wird unter Stickstoffatmosphäre in einem aprotischen oder unpolaren organischen Lösungsmittel, wie beispielsweise Diethylether, Methyl-tert.-butylether, Tetrahydrofuran, Dioxan, Toluol, Xylol, Methylenchlorid oder Chloroform gelöst und zu einer unter Stickstoff-Atmosphäre bereiteten Lösung einer einfach molaren bis zu 60-prozentig überschüssigen Menge Oxalylchlorid in einem aprotischen oder unpolaren Lösungsmittel, wie z. B. in Diethylether, Methyl-tert.butylether, Tetrahydrofuran, Dioxan, Toluol, Xylol, Methylenchlorid gegeben, wobei die Temperatur zwischen –5°C und 20°C gehalten wird. Man erhitzt sodann die Reaktionslösung bei einer Temperatur zwischen 10°C und 130°C, vorzugsweise zwischen 20°C und 80°C, besonders zwischen 30°C und 50°C für einen Zeitraum von 30 Minuten bis zu 5 Stunden und dampft anschließend das Lösungsmittel ab. Der verbleibende Rückstand des auf diese Weise gebildeten Indolyl-3-glyoxylsäurechlorids wird in einem aprotischen Lösungsmittel wie z. B. Tetrahydrofuran, Dioxan, Diethylether, Toluol oder auch in einem dipolar aprotischen Lösungsmittel, wie z. B. Dimethylformamid, Dimethylacetamid oder Dimethylsulfoxid gelöst, auf eine Temperatur zwischen 10°C und –15°C, vorzugsweise zwischen –5°C und 0°C, gekühlt und in Gegenwart eines Säurefängers

mit einer Lösung des primären oder sekundären Amins in einem Verdünnungsmittel versetzt. Als Verdünnungsmittel kommen die oben zur Auflösung des Indolyl-3-glyoxylsäurechlorids verwendeten Lösungsmittel in Frage. Als Säurefänger finden Triethylamin, Pyridin, Dimethylaminopyridin, bas. Ionenaustauscher, Natriumcarbonat, Kaliumcarbonat, pulverisiertes Kaliumhydroxid sowie überschüssiges, zur Reaktion eingesetztes, primäres oder sekundäres Amin Verwendung. Die Reaktion findet bei einer Temperatur von 0°C bis 120°C, vorzugsweise bei 20–80°C, besonders zwischen 40°C und 60°C statt. Nach 1–3stündiger Reaktionszeit und 24stündigem Stehen bei Raumtemperatur wird das Hydrochlorid des Säurefängers filtriert, das Filtrat i. Vak. eingeengt und der Rückstand aus einem organischen Lösungsmittel umkristallisiert oder durch Säulenchromatographie über Kieselgel oder Aluminiumoxid gereinigt. Als Laufmittel findet z. B. ein Gemisch aus Dichlormethan und Ethanol (95 : 5, Vol/Vol) Verwendung.

Ausführungsbeispiele

Gemäß dieser allgemeinen Vorschrift für die Stufen 1 und 2, denen das Syntheseschema 1 zugrundeliegt, wurden folgende Verbindungen synthetisiert, die unter Angabe der jeweiligen chemischen Bezeichnung aus der nachfolgenden Übersicht hervorgehen. In den Tabellen 1a–j auf den Seiten A–J sind aus der allgemeinen Formel 1 und den Substituenten R_1–R_4 und Z die Strukturen dieser Verbindungen und ihre Schmelzpunkte zu ersehen:

Beispiel 1

N-(Pyridin-4-yl)-[1-(4-fluorbenzyl)-indol-3-yl]glyoxylamid (D 24241)

1. Stufe

1-(4-Fluorbenzyl)-indol

In eine Mischung von 2,64 g Natriumhydrid (0,11 Mol, Mineralölsuspension) in 100 ml Dimethylsulfoxid wird eine Lösung von 11,72 g (0,1 Mol) Indol in 50 ml Dimethylsulfoxid gegeben. Man erhitzt 1,5 Stunden auf 60°C, läßt danach abkühlen und tropft 15,9 g (0,11 Mol) 4-Fluorbenzylchlorid zu. Die Lösung wird auf 60°C erwärmt, über Nacht stehengelassen und sodann unter Rühren in 400 ml Wasser gegossen. Man extrahiert mehrmals mit insgesamt 150 ml Methylenchlorid, trocknet die organische Phase mit wasserfreiem Natriumsulfat, filtriert und engt das Filtrat i. Vak. ein. Der Rückstand wird i. Hochvakuum destilliert: 21,0 g (96% d. Th.) Sdp. (0,5 mm): 140°C.

2. Stufe

N-(Pyridin-4-yl)-[1-(4-fluorbenzyl)-indol-3-yl]glyoxylamid (D 24241)

Zu einer Lösung von 2,25 ml Oxalylchlorid in 25 ml Ether wird bei 0°C und unter N_2 tropfenweise eine Lösung von 4,75 g (21,1 mMol) 1-(4-Fluorbenzyl)-indol in 25 ml Ether gegeben. Man erhitzt 2 Stunden zum Rückfluß und dampft anschließend das Lösungsmittel ab. Sodann wurden zum Rückstand 50 ml Tetrahydrofuran zugefügt, die Lösung auf –5°C abgekühlt und tropfenweise mit einer Lösung von 4,66 g (49,5 mMol) 4-Aminopyridin in 200 ml THF versetzt. Man erhitzt 3 Stunden zum Rückfluß und läßt über Nacht bei Raumtemperatur stehen. Das 4-Aminopyridin Hydrochlorid wird abgesaugt, der Niederschlag mit THF gewaschen, das Filtrat i. Vak. eingeengt und der Rückstand aus Essigester umkristallisiert.
Ausbeute: 7,09 g (90% d. Th.)
Schmelzpunkt: 225–226°C
Elementaranalyse:
berechnet:
C 70,77; H 4,32; N 11,25;
gefunden:
C 71,09; H 4,36; N 11,26.

Beispiel 2, D 24242 N-(Pyridin-4-yl)-(1-methyl-indol-3-yl)glyoxylamid
Beispiel 3, D 24834 N-(Pyridin-3-yl)-[1-(4-fluorbenzyl)-indol-3-yl]-glyoxylamid
Beispiel 4, D 24835 N-(Pyridin-3-yl)-(1-benzylindol-3-yl)-glyoxylamid
Beispiel 5, D 24836 N-(Pyridin-3-yl)-[1-(2-chlorbenzyl)-indol-3-yl]-glyoxylamid
Beispiel 6, D 24840 N-(4-Fluorphenyl)-[1-(4-fluorbenzyl)-indol-3-yl]-glyoxylamid
Beispiel 7, D 24841 N-(4-Nitrophenyl)-[1-(4-fluorbenzyl)-indol-3-yl]-glyoxylamid
Beispiel 8, D 24842 N-(2-Chlorpyridin-3-yl)-[1-(4-fluorbenzyl)-indol-3-yl]-glyoxylamid
Beispiel 9, D 24843 N-(Pyridin-4-yl)-(1-benzylindol-3-yl)-glyoxylamid
Beispiel 10, D 24848 N-(Pyridin-4-yl)-[1-(3-pyridylmethyl)-indol-3-yl]-glyoxylamid
Beispiel 11, D 24849 N-(4-Fluorphenyl)-[1-(2-pyridylmethyl)-indol-3-yl]-glyoxylamid
Beispiel 12, D 24850 N-(4-Fluorphenyl)-[1-(3-pyridylmethyl)-indol-3-yl]-glyoxylamid
Beispiel 13, D 24851 N-(Pyridin-4-yl)-[1-(4-chlorbenzyl)-indol-3-yl]-glyoxylamid
Beispiel 14, D 24852 N-(Pyridin-4-yl)-[1-(2-chlorbenzyl)-indol-3-yl]-glyoxylamid
Beispiel 15, D 24853 N-(Pyridin-2-yl)-[1-(4-fluorbenzyl)-indol-3-yl]-glyoxylamid
Beispiel 16, D 24847 N-(Pyridin-4-yl)-[1-(2-pyridylmethyl)-indol-3-yl]-glyoxylamid
Beispiel 17, D 24858 (4-Phenyl-piperazin-1-yl)-[1-(4-fluorbenzyl)-indol-3-yl]-glyoxylamid
Beispiel 18, D 24854 N-(Pyridin-2-yl)-(1-benzyl-indol-3-yl)-glyoxylamid
Beispiel 19, D 25421 N-(Pyridin-4-yl)-[1-(4-fluorbenzyl)-6-ethoxycarbonylamino-indol-3-yl]-glyoxylamid

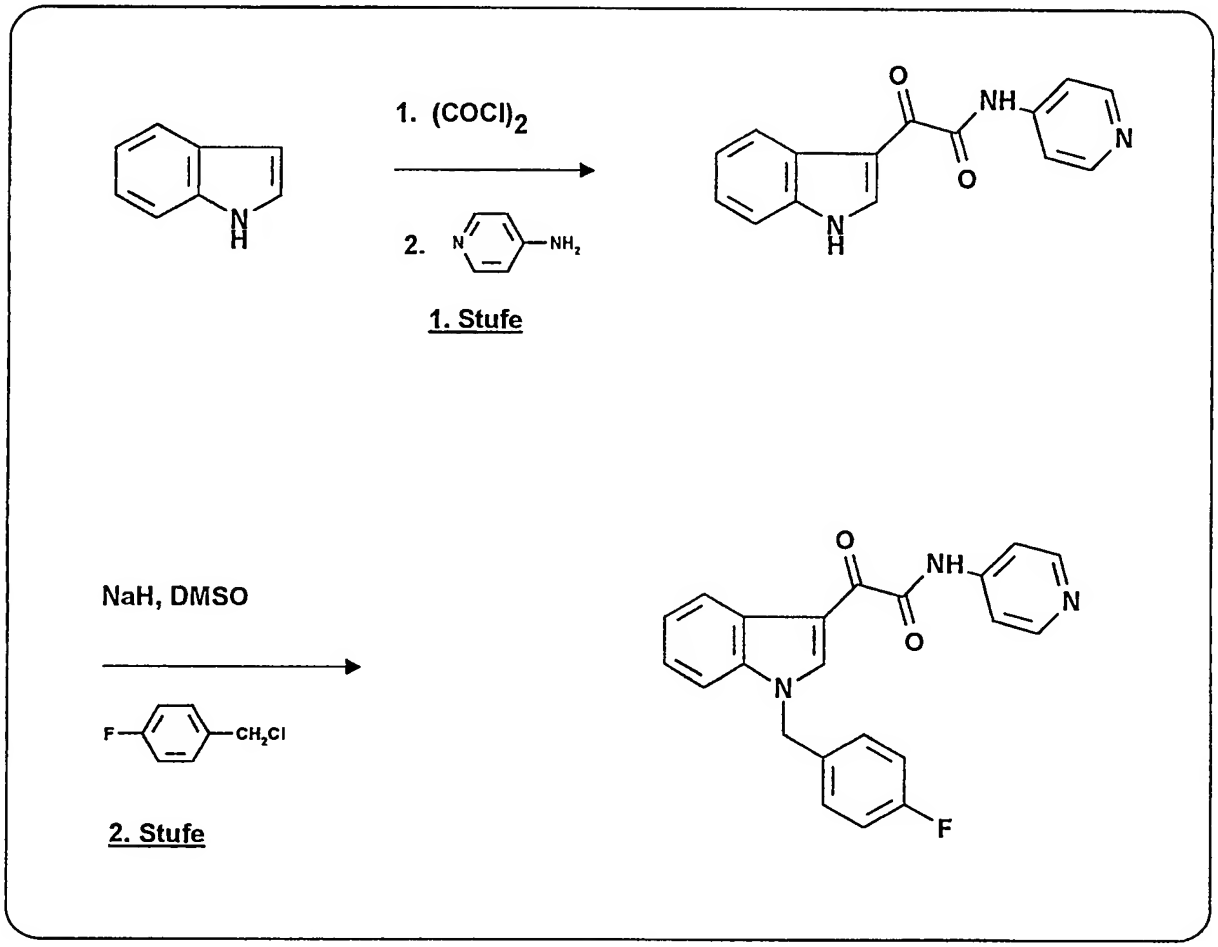
Beispiel 20, D 25422 N-(Pyridin-4-yl)-[1-(4-fluorbenzyl)-5-ethoxycarbonylamino-indol-3-yl]-glyoxylamid
Beispiel 21, D 25423 N-(Pyridin-4-yl)-[1-(4-fluorbenzyl)-6-cyclopentyloxycarbonylamino-indol-3-yl]-glyoxylamid
Beispiel 22, D 25420 4-(Pyridin-4-yl)-piperazin-1-yl)-[1-(4-fluorbenzyl)-indol-3-yl]-glyoxylamid
Beispiel 23, D 24866 N-(3,4,5-Trimethoxybenzyl)-N-(allylaminocarbonyl-2-methyl-prop-1-yl)-[1-(4-fluorbenzyl)-indol-3-yl]-glyoxylamid
Beispiel 24 N-(Pyridin-4-yl)-[1-(4-fluorbenzyl)-5-methoxy-indol-3-yl]-glyoxylamid
Beispiel 25 N-(Pyridin-4-yl)-[1-(4-fluorbenzyl)-5-ethoxycarbonylamino-methylindol-3-yl]-glyoxylamid.

Ausgangsstufen für die nach Syntheseschema 1 hergestellten Verbindungen der allgemeinen Formel 1 die aus Tabelle 1 hervorgehen

Für die Syntheseendstufen D 24241, D 24242, D 24834, D 24835, D 24;836, D 24840, D 24841, D 24842, D 24843, D 24848, D 24849, D 24850, D 24851, D 24852, D 24853, D 24847, D 24858, D 24854, D 25420, D 25422, D 25421, D 25423 sind alle Vorstufen käuflich.

Weiterhin sind die Verbindungen der allgemeinen Formel 1 mit Z = O, R_1 = Aryl, Aralkyl, Heteroaryl, Heteroaralkyl und der Allylaminocarbonyl-2-methyl-prop-1-yl-Gruppe sowie R_2 = alkyl,aralkyl und der Heteroaralkyl-Gruppe auch nach dem Syntheseweg des Schemas 2 erhältlich:

Schema 2



Nach dem vorliegenden Schema 2 wurden die Verbindungen D 24241, D24841, D 24840 und D 24834 (2. Stufe des Reaktionsschemas 2, s. auch Tabelle 1) sowie deren jeweilige Vorstufen D 24825, D 24831, D 24832 und D 24833 (1. Stufe des Reaktionsschemas 2, s. auch Tabelle 2 auf der Seite K) erhalten.

N-(Pyridin-4-yl)-[1-(4-fluorbenzyl)-indol-3-yl]glyoxylamid (D 24241)

1. Stufe

N-(Pyridin-4-yl)-(indol-3-yl)glyoxylamid

Zu einer Lösung von 9 ml Oxalylchlorid in 100 ml wasserfreiem Ether wird tropfenweise bei 0°C eine Lösung von 10 g (85.3 mMol) Indol in 100 ml Ether zugegeben. Man hält das Gemisch 3 Stunden unter Rückfluß. Sodann wird bei −5°C eine Suspension von 12 g (127,9 mMol) 4-Aminopyridin in 500 ml Tetrahydrofuran zugetropft, das Reaktionsgemisch unter Rühren 3 Stunden auf Rückflußtemperatur erhitzt und über Nacht bei Raumtemp. stehengelassen. Man fil-

trierte, behandelte den Niederschlag mit Wasser und reinigte die getrocknete Verbindung über eine Kieselgelsäule (Kieselgel 60, Fa. Merck AG, Darmstadt) unter Anwendung des Elutionsmittels Methylenchlorid/Ethanol (10 : 1, v/v).
Ausbeute: 9,8 g (43,3% d. Th.)
Fp.: ab 250°C.

2. Stufe

N-(Pyridin-4-yl)-[1-(4-fluorbenzyl)-indol-3-yl]glyoxylamid (D 24241)

Das nach der 1. Stufe erhaltene N-(Pyridin-4-yl)-(indol-3-yl)glyoxylamid wird gemäß der "Benzylierungsvorschrift" (Seite 5) mit 4-Fluorbenzylchlorid umgesetzt und die erhaltene Verbindung D 24241 isoliert.
Ausbeute: 41% d. Th.
Schmp.: 224–225°C
Elementaranalyse:
Berechnet:
C 70,77; H 4,32; N 11,25;
Gefunden:
C 70,98; H 4,40; N 11,49.

Allgemeine Vorschrift zur Darstellung der Verbindungen der allgemeinen Formel 1 gemäß Schema 2

1. Stufe

Zu einer unter Stickstoffatmosphäre bereiteten Lösung einer einfach molaren bis zu 60% überschüssigen Menge Oxalylchlorid in einem aprotischen oder unpolaren Lösungsmittel, wie z. B. in Diethylether, Methyl-tert.-butylether, Tetrahydrofuran, Dioxan oder auch Dichlormethan, wird bei einer Temperatur zwischen –5°C und +5°C tropfenweise das in einem Lösungsmittel, wie z. B. oben für Oxalylchlorid angegeben, gelöste Indol-Derivat, das unsubstituiert oder an C-2 bzw. im Phenylring substituiert sein kann, zugegeben. Man erhitzt sodann die Reaktionslösung für 1 bis zu 5 Stunden auf eine Temperatur zwischen 10°C und 120°C, vorzugsweise zwischen 20°C und 80°C, besonders zwischen 30°C und 60°C und dampft anschließend das Lösungsmittel ab. Der verbleibende Rückstand des (Indol-3-yl)glyoxylsäurechlorids wird in einem aprotischen Lösungsmittel, wie z. B. Tetrahydrofuran, Dioxan, Diethylether, Toluol oder auch in einem dipolar aprotischen Lösungsmittel, wie z. B. Dimethylformamid, Dimethylacetamid oder Dimethylsulfoxid gelöst bzw. suspendiert, auf eine Temperatur zwischen –10°C und +10°C, vorzugsweise auf –5°C bis 0°C gekühlt und in Gegenwart eines Säurefängers mit einer Lösung des primären oder sekundären Amins in einem Verdünnungsmittel versetzt. Als Verdünnungsmittel kommen die zur Auflösung des "Indolyl-3-glyoxylsäurechlorids" verwendeten Lösungsmittel in Frage. Als Säurefänger finden Triethylamin, Pyridin, Dimethylaminopyridin, bas. Ionenaustauscher, Natriumcarbonat, Kaliumcarbonat, pulverisiertes Kaliumhydroxid sowie überschüssiges, zur Reaktion eingesetztes primäres oder sekundäres Amin Verwendung. Die Reaktion findet bei einer Temperatur von 0°C bis 120°C, vorzugsweise bei 20–80°C, besonders zwischen 40°C und 60°C statt. Nach 1–4stündiger Reaktionszeit und 24stündigem Stehen bei Raumtemperatur wird filtriert,der Niederschlag mit Wasser digeriert, abgesaugt und i. Vak. getrocknet. Man reinigt die gewünschte Verbindung durch Umkristallisation in einem organischen Lösungsmittel oder durch Säulenchromatographie an Kieselgel oder Aluminiumoxid. Als Laufmittel findet z. B. ein Gemisch aus Dichlormethan und Ethanol (10 : 1, vol/vol) Verwendung.

2. Stufe

Das nach obenstehender Vorschrift der 1. Stufe erhaltene "Indol-3-yl-glyoxylamid" wird in einem protischen, dipolar aprotischen oder unpolaren organischen Lösungsmittel, wie z. B. in Isopropanol, Tetrahydrofuran, Dimethylsulfoxid, Dimethylformamid, Dimethylacetamid, N-Methylpyrrolidon, Dioxan, Toluol oder Methylenchlorid gelöst und tropfenweise zu einer in einem Dreihalskolben unter N_2-Atmosphäre vorbereiten molaren oder überschüssig eingesetzten Suspension einer Base, wie z. B. Natriumhydrid, pulverisiertes Kaliumhydroxid, Kalium-tert.-butylat, Dimethylaminopyridin oder Natriumamid in einem geeigneten Lösungsmittel gegeben. Sodann gibt man das gewünschte Alkyl-, Aralkyl- bzw. Heteroaralkylhalogenid entweder unverdünnt oder in einem Verdünnungsmittel, das z. B. auch zur Lösung des "Indol-3-yl-glyoxylamids" verwendet wurde, gegebenenfalls unter Zusatz eines Katalysators, wie z. B. Kupfer, zu und läßt einige Zeit, z. B. 30 Minuten bis 12 Stunden, reagieren und hält die Temperatur innerhalb eines Bereichs zwischen 0°C und 120°C, vorzugsweise zwischen 30°C und 80°C, besonders zwischen 50 und 70°C. Nach Beendigung der Reaktion wird das Reaktionsgemisch in Wasser gegeben, die Lösung z. B. mit Diethylether, Dichlormethan, Chloroform, Methyl-tert.-butylether, Tetrahydrofuran bzw. n-Butanol extrahiert und die jeweils erhaltene organische Phase mit wasserfreiem Natriumsulfat getrocknet.

Man engt die organische Phase im Vakuum ein, kristallisiert den verbleibenden Rückstand durch Anreiben bzw. reinigt den öligen Rückstand durch Destillation oder durch Säulen- bzw. Flashchromatographie an Kieselgel oder Aluminiumoxid. Als Laufmittel dient beispielsweise ein Gemisch aus Methylenchlorid und Diethylether im Verhältnis 8 : 2 (Vo/Vol) oder ein Gemisch aus Methylenchlorid und Ethanol im Verhältnis 9 : 1 (V/V).

Gemäß dieser allgemeinen Vorschrift für die Stufen 1 und 2, denen das Syntheseschema 2 zugrundeliegt, wurden die Verbindungen D 24241, D 24841, D 24840 und D 24834 synthetisiert, die auch schon gemäß des Syntheseablaufs des Reaktionsschemas 1 dargestellt wurden und aus Tabelle 1 hervorgehen. Die diesbezüglichen Vorstufen dieser Verbindungen sind aus Tabelle 2 auf Seite K und L ersichtlich.

Die Verbindungen zeigen eine gute dosisabhängige Antitumorwirkung in den folgenden pharmakologischen Modellen:

Die Indole besonders D-24851 und D-24241 sind zuerst im XTT-Proliferationstest-/Zytotoxizitätstest aufgefallen (Tab. 3 und Tab. 3a). In diesem Testsystem wird der Einfluß von Substanzen auf das Proliferationsverhalten von Tumorzellinien untersucht. Dabei wird das zytotoxische Potential dieser Substanzen erfaßt. Die Testmethode ist bei Scudiero et al. 1988, Cancer Res. 48, 4827 beschrieben.

Es wurden bei den Untersuchungen folgende Tumorzellinien eingesetzt:
Die KB-Zellinie ein epidermales Karzinom der Mundhöhle die L1210-Zellinie eine lymphatische Leukämie der Maus die LNCAP-Zellinie ein Prostatakarzinom und
die SK-OV-3 Zellinie ein Ovarial-karzinom.

In allen vier Tumorzellinien war eine große Anzahl von verschiedenen Indolen wirksam. Die stärksten Wirkungen zeigten D-24851 und D-24241, wobei D-24851 wirksamer war als D-24241 (Tab. 3 und 4).

In weiteren vergleichenden Untersuchungen mit D-24851 und D-24241 im Hohlfaser Assay an der Nacktmaus und an der L1210 (Maus) konnte bei beiden Verbindungen eine starke dosisabhängige Antitumorwirkung beobachte werden (Tab. 3 und 5). Im Hohlfaser Assay waren beide Verbindungen nahezu gleich stark wirksam, während an der L1210 D-24851 nach peroraler und intraperitonealer Gabe deutlich stärker wirksam war als D-24241. Im Vergleich zu den auf dem Markt befindlichen Antitumor-Substanzen ist D-24851 im Leukämiemodell in vielen Fällen deutlich stärker wirksam als die bekannten Vergleichsubstanzen (Tab. 5).

Ein weiterer großer Vorteil von D-24851 im Vergleich zu den auf dem Markt befindlichen Antitumor-Substanzen ist die geringe Toxizität der Verbindung (Tab. 3 und 5). Mit LD 50 Werten von 1000 mg/kg p.o. und > 1000 mg/kg i.p. verfügt die Verbindung über eine große therapeutische Breite.

Weiterhin konnte nach Gabe von D-24851 keine DNA-Fragmentierung beobachtet werden. Auch im Haematopoese Versuch wurden keine der untersuchten Blutparameter durch die intraperitoneale Gabe von D-24851 verändert.

In einem weiteren Chemotherapiemodell dem Dunning-Tumor an der Ratte konnte nach mehrmaliger peroraler Gabe von D-24851 ein Tumorwachstumsstop und bei einigen Tieren sogar eine Tumorregression beobachtet werden.

Im KB-Versuch an der Nacktmaus konnte ebenfalls nach Gabe der beiden Indole D-24851 und D-24241 eine Antitumorwirkung beobachtet werden (Tab. 3, 3a und 4).

Bei den Untersuchungen mit der Tumorzellinie L1210, eine lymphatische Leukämie der Maus, zeigte sich nach intraparitonealer bzw. peroraler Gabe von D 24851 mit 100 und 147 mg/kg mehrfach-Gabe eine deutliche dosisabhängige Verlängerung der Überlebenszeit (**Fig.** 1a und **Fig.** 1b).

Aufgrund der guten therapeutischen Breite, die experimentell nachgewiesen wurde, kann die Wirksubstanz höher als handelsübliche Tumorpharmaka dosiert werden.

Ohne mit der nach folgenden Angabe den Umfang der Erfindung einzuschränken zu wollen ist zu sagen, daß Dosierungen ab etwa 20 mg bis zu 500 mg täglich oral möglich sind. Bei intravenöser Gabe als Injektion oder als Infusion können bis zu 250 mg/Tag oder mehr je nach Körpergewicht des Patienten und individueller Verträglichkeit verabreicht werden.

Tabelle 3

Zusammenstellung D-24851 gemäß Beispiel 13

D-24851 N-(Pyridin-4-yl)-[1-(4-chlorbenzyl)-indol-3-yl]glyoxylamid

Modell	Result.	SK-OV-3	KB	L1210	LNCaP	MCF-7	Tox
XTT (µg/ml)	EC_{50}	≈ 0.03	≈ 0.017	≈ 0.017	≈ 0.03		
1x ip (mg/kg)	DL_{50}						= 1000
1x per os (mg/kg)	DL_{50}						> 1000
Hohlfaser intraperitoneal 4 x 46 mg/kg ip	% INH		keine Wirkung	56		38	
Hohlfaser intraperitoneal 4 x 147 mg/kg ip	% INH		12	60		68	
Hohlfaser subcutan 4 x 46 mg/kg ip	% INH		44	keine Wirkung		47	
Hohlfaser subcutan 4 x 147 mg/kg ip	% INH		35	67		68	
In Vivo:							
1 x 681 mg/kg ip 1 x 464 mg/kg ip	% ILS			0 18			
4 x 215 mg/kg ip 4 x 147 mg/kg ip	% ILS			13 94			
7 x 100 mg/kg ip 7 x 147 mg/kg ip	% ILS			35 59			
1 x 681 mg/kg po 4 x 215 mg/kg po 7 x 100 mg/kg po 7 x 147 mg/kg po	% ILS			22 31 63 75			
7 x 46 mg/kg ip 2 x 215 mg/kg po	% WHI		33 18				

Tabelle 3a

Substanz gemäß Beispiel	Tumorzellen XTT			
(D-Nummer)	KB	L 1210	LNCAP	SK-OV-3
	EC_{50} [µg/ml]	EC_{50} [µg/ml]	EC_{50} [µg/ml]	EC_{50} [µg/ml]
1 (D 24241)	0,020	0,170	>31,600	0,170
3 (D 24834)	1,75	1,75	9,250	1,750
4 (D 24835)	17,5	1,750	>31,6	9,200
6 (D 24840)	3,100	1,750	>31,6	17,5
9 (D 24843)	0,050	0,090	3,240	1,750
10 (D 24848)	4,060	1,75	>31,6	7,220
11 (D 24849)	4,590	1,750	17,500	4,250
12 (D 24850)	>31,6	0,017	>31,6	>31,6
13 (D 24851)	0,017	0,017	0,030	0,030
14 (D 24852)	1,75	1,75	17,5	2,58
15 (D 24853)	>31,6	3,1	>31,6	>31,6
16 (D 24847)	4,59	1,75	17,500	4,250
Tabelle 2 (D 24831)	17,5	17,5	17,5	17,5

Weitere tierexperimentelle Ergebnisse:

Am Dunning Tumor konnte nach Gabe von 7×100 mg/kg und 7×147 mg/kg p.o. D-24851 ein Tumorwachstumsstop, bei einigen Tieren sogar eine Tumorregression beobachtet werden.

Die Testung der kristallinen Form brachte im Vergleich zur ursprünglichen Form keine Unterschiede.

D-24851 verursacht keine DNA-Fragmentierung.

Im Haematopoese Versuch wurde keiner der untersuchten Blutparameter durch die intraperitoneale Gabe von D-24851 verändert.

Tabelle 4

D 24241 N-(Pyridin-4-yl)-[1-(4-fluorbenzyl)-indol-3-yl]glyoxylamid gemäß Beispiel 1

Modell	Result.	SK-OV-3	KB	L1210	LNCaP	MCF-7	Tox
XTT (µg/ml)	EC_{50}	≈ 0.17	≈ 0.02	≈ 0.17	>31.6		
1x ip (mg/kg)	DL_{50}						≈ 158
1x per os (mg/kg)	DL_{50}						> 1000
Hohlfaser intraperitoneal 4 x 15.8 mg/kg ip	% INH		46	43		keine Wirkung	
Hohlfaser subcutan 4 x 15.8 mg/kg ip	% INH		81	68		33	
In Vivo:							
1 x 14.7 mg/kg ip	% ILS			keine Wirkung			
1 x 30 mg/kg per os	% ILS			keine Wirkung			
1 x 464 mg/kg per os	% ILS			44			
4 x 30 mg/kg per os	% ILS			keine Wirkung			
6 x 30 mg/kg per os	% ILS			keine Wirkung			
14 x 30 mg/kg per os	% ILS			keine Wirkung			
19 x 50 mg/kg per os	% ILS			50			
2 x 46.4 mg/kg ip	%WHI		22				
4 x 21.5 mg/kg ip	%WHI		keine Wirkung				
2 x 215 mg/kg po	%WHI		47				

Tabelle 5

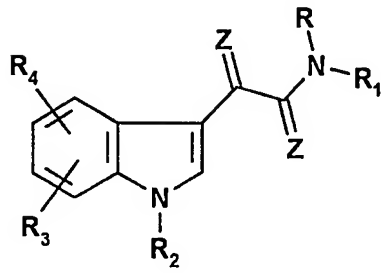
Vergleich der Antitumorwirkung von D-24851 bzw. D-24241 mit Standardverbindungen

Substanz	Tox. mg/kg	L1210 mg/kg	XTT EC 50 (µg/ml)
D-24851	≈1000 i.p.	4x 147 i.p. 94% ILS	KB ≈ 0.017 L1210 ≈ 0.017 SKOV3 ≈ 0.03 LNCAP ≈ 0.03
D-24241	≈ 158 i.p.	19x 50 p.o. 50% ILS	KB ≈ 0.02 L1210 ≈ 0.17 SKOV3 ≈ 0.17 LNCAP > 31.6
Mitoxantron	16 i.v.	1x 4.64 i.v. 144% ILS	KB ~ 0.174 L1210 < 0.0003 SKOV3 ~0.174 LNCAP ~0.017
5-Fluoruracil	----	1x 147 i.p. 72% ILS 4x 68.1 i.p. 83% ILS	------
Methotrexat	-----	1x 53.7 i.p. 39% ILS	KB ~ 0.007 L1210 n.d. SKOV3 > 31.6 LNCAP n.d.

Substanz	Tox. mg/kg	L1210 mg/kg	XTT EC 50 (µg/ml)
Etoposid	≈158.0 i.p > 68.1 i.v.	1x 46.4 i.p. 56% ILS	------
Ratjadon	~ 16.0 i.p. ~ 30.0 i.v.	1x 1.47 i.p. 22% ILS	KB < 0.003 L1210 < 0.003 SKOV3<0.003 LNCAP<0.003
Epothilon B	≈100.0 i.p.	1x 10 i.p. 44% ILS	KB ~0.0002 L1210~0.0017 SKOV3~0.0031 LNCAP~0.014
Taxol	≈158 i.p.	1x 14.7 i.v. 22 % ILS 1x 46.4 i.v. 61 % ILS	KB < 0.003 L1210 < 0.003 SKOV3< 0.003 LNCAP< 0.003
Vincristin	≈ 3.0 i.v.	1x1.0 i.p. 29% ILS	KB < 0.001 L1210 0.004 SKOV3 0.003 LNCAP 0.004
Adriamycin	≈ 27.0 i.v.	1x14,7 i.v. 111% ILS	KB 0.15 L1210 0.174 SKOV3 0.089 LNCAP 0.17
Cisplatin	≈ 16.0 i.p. ≈ 73.0 p.o.	1x3.16 i.p. 38.9% ILS	L1210 0.30
Carboplatin	≈158.0 i.p. ≈841.0 p.o.	1x100 i.p. 41% ILS	------
Lobaplatin	≈ 34.0 i.p.	1x14.7 i.p. 55.0% ILS	------
Cyclophosphamid	≈340.7 i.v.	1x46.4 i.v. 40% ILS	------
Ifosfamid	≈732 i.p.	1x316 i.p. 89% ILS	------
Miltefosin	≈ 46.4 i.p. ≈464-1000 p.o.	keine Wkg.	-------

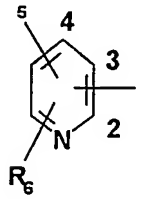
Patentansprüche

1. N-substituierte Indol-3-gloxylamide der allgemeinen Formel 1 zur Verwendung als Antitumormittel,



Formel 1

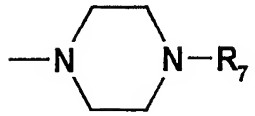
wobei die Reste R, R_1, R_2, R_3, R_4 und Z folgende Bedeutung haben:
R = Wasserstoff, (C_1-C_6)-Alkyl, wobei die Alkylgruppe ein- oder mehrfach durch den Phenylring substituiert sein kann und dieser Phenylring seinerseits ein- oder mehrfach durch Halogen, (C_1-C_6)-Alkyl, (C_3-C_7)-Cycloalkyl, durch Carboxylgruppen, mit C_1-C_6-Alkanolen veresterte Carboxylgruppen, Trifluormethylgruppen, Hydroxylgruppen, Methoxygruppen, Ethoxygruppen, Benzyloxygruppen sowie durch eine im Phenylteil ein- oder mehrfach mit (C_1-C_6)-Alkylgruppen, Halogenatomen oder Trifluormethylgruppen substituierte Benzylgruppe substituiert sein kann,
R steht ferner für die Benzyloxycarbonyl-Gruppe (Z-Gruppe) und für den tertiär-Butoxycarbonylrest (Boc-Rest), weiterhin für die Acetylgruppe.
R_1 kann den Phenylring, der ein- oder mehrfach mit (C_1-C_6)-Alkyl, (C_1-C_6)-Alkoxy, Cyano, Halogen, Trifluormethyl, Hydroxy, Benzyloxy, Nitro, Amino, (C_1-C_6)-Alkylamino, (C_1-C_6)-Alkoxycarbonylamino und mit der Carboxylgruppe bzw. mit der mit C_1-C_6-Alkanolen veresterten Carboxylgruppe substituiert ist, oder ein Pyridin-Gerüst der Formel 2 und deren N-Oxid



Formel 2

bedeuten und dessen N-Oxid, wobei das Pyridin-Gerüst wahlweise an den Ringkohlenstoff Atomen 2, 3 und 4 gebunden ist und mit den Substituenten R_5 und R_6 substituiert sein kann. Die Reste R_5 und R_6 können gleich oder verschieden sein und die Bedeutung (C_1-C_6)-Alkyl sowie die Bedeutung (C_3-C_7)-Cycloalkyl, (C_1-C_6)-Alkoxy, Nitro, Amino, Hydroxy, Halogen und Trifluormethyl besitzen und ferner den Ethoxycarbonylamino-Rest sowie die Gruppe Carboxyalkyloxy darstellen, bei dem die Alkylgruppe über 1–4 C-Atome verfügen kann.
R_1 kann ferner ein 2- bzw. 4-Pyrimidinyl-Heterocyclus sein, wobei der 2-Pyrimidinyl-Ring ein- oder mehrfach mit der Methylgruppe substituiert sein kann, weiterhin das mit (C_1-C_6)-Alkyl, Halogen, der Nitrogruppe, der Aminogruppe und dem (C_1-C_6)-Alkyl- amino-Rest substituierte 2-, 3-, und 4- und 8-Chinolylgerüst bedeuten, eine 2-, 3- und 4-Chinolylmethylgruppe darstellen, wobei die Ringkohlenstoffe des Pyridylmethylrestes der Chinolylgruppe und des Chinolylmethyl-Restes mit (C_1-C_6)-Alkyl$_1$ (C_1-C_6)-Alkoxy, Nitro, Amino und (C_1-C_6)-Alkoxycarbonylamino substituiert sein können.
R_1 kann weiterhin für den Fall, daß R = Wasserstoff, die Methyl -oder Benzylgruppe sowie den Benzyloxycarbonyl-Rest (Z-Rest), den tert.-Butoxycarbonyl-Rest (BOC-Rest) und die Acetylgruppe darstellt, die folgenden Reste bedeuten:–
CH_2COOH; $-CH(CH_3)-COOH$; $-(CH_3)_2-CH-(CH_2)_2-CH-COO-$; $H_3C-H_2C-CH(CH_3)-CH(COOH)-$; $HO-H_2C-CH(COOH)-$; Phenyl-CH_2-CH(COOH)-; (4-Imidazolyl)-CH_2-CH-(COOH)-; $HN=C(NH_2)-N$ H-$(CH_2)_3$-CH(COOH)-; $H_2N-(CH_2)_4-CH(COOH)-$; $H_2N-CO-CH_2-CH-(COOH)-$; $HOOC-(CH_2)_2-CH(COOH)-$;
R_1 kann weiterhin für den Fall, daß R Wasserstoff, die Z-Gruppe, den BOC-Rest, die Acetyl- oder die Benzylgruppe bedeuten, der Säurerest einer natürlichen oder unnatürlichen Aminosäure sein, z. B. den α-Glycyl-, den α-Sarkosyl-, den α-Alanyl-, den α-Leucyl-, den α-iso-Leucyl-, den α-Seryl-, den α-Phenylalanyl-, den α-Histidyl-, den α-Prolyl-, den α-Arginyl-, den α-Lysyl-, den α-Asparagyl- und den α-Glutamyl-Rest darstellen, wobei die Aminogruppen der jeweiligen Aminosäuren ungeschützt vorliegen oder geschützt sein können. Als Schutzgruppe der Aminofunktion kommen der Carbobenzoxy-Rest (Z-Rest) und der tert.-Butoxycarbonyl-Rest (BOC-Rest) sowie die Acetylgruppe in Frage. Im Fall des für R_1 beanspruchten Asparagyl- und Glutamylrestes liegt die zweite, nicht gebundene Carboxylgruppe als freie Carboxylgruppe oder in Form eines Esters mit C_1-C_6-Alkanolen, z. B. als Methyl-, Ethyl- bzw. als tert.-Butylester vor.
Weiterhin kann R_1 die Allylaminocarbonyl-2-methyl-prop-1-yl-Gruppe bedeuten.
R und R_1 können ferner zusammen mit dem Stickstoff-Atom, an das sie gebunden sind, einen Piperazinring der For-

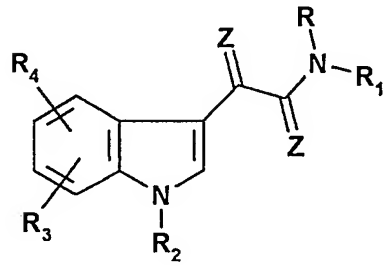
mel 3 oder einen Homopiperazinring bilden, sofern R_1 eine Aminoalkylengruppe darstellt, bei dem



Formel 3

R_7 einen Alkylrest darstellt, einen Phenylring bedeutet, der ein- oder mehrfach mit (C_1-C_6)-Alkyl, (C_1-C_6)-Alkoxy, Halogen, der Nitrogruppe, der Aminofunktion und mit der (C_1-C_6)-Alkylaminogruppe substituiert sein kann. R_7 bedeutet ferner die Benzhydryl-Gruppe und die Bis-p-fluorbenzylhydryl-Gruppe.
R_2 kann Wasserstoff und die (C_1-C_6)-Alkyl-Gruppe bedeuten, wobei die Alkylgruppe ein- oder mehrfach durch Halogen und Phenyl substituiert ist, das seinerseits ein- oder mehrfach durch Halogen, (C_1-C_6)-Alkyl, (C_3-C_7)-Cycloalkyl, Carboxylgruppen mit C_1-C_6-Alkanolen veresterten Carboxylgruppen, Trifluormethylgruppen, Hydroxylgruppen, Methoxygruppen, Ethoxygruppen oder Benzyloxygruppen substituiert sein kann. Die für R_2 geltende (C_1-C_6)-Alkyl-Gruppe kann ferner durch die 2-Chinolylgruppe und das 2-,3- und 4-Pyridyl-Gerüst substituiert sein, die beide jeweils ein- oder mehrfach durch Halogen, (C_1-C_4)-Alkylgruppen oder (C_1-C_4)-Alkoxy-gruppen substituiert sein können. R_2 steht ferner für den Aroyl-Rest, wobei der diesem Rest zugrunde liegende Arylteil den Phenylring darstellt, der ein- oder mehrfach durch Halogen, (C_1-C_6)-Alkyl, (C_3-C_7)-Cycloalkyl, Carboxylgruppen mit C_1-C_6-Alkanolen veresterte Carboxylgruppen, Trifluormethylgruppen, Hydroxylgruppen, Methoxygruppen, Ethoxygruppen oder Benzyloxygruppen substituiert sein kann.
R_3 und R_4 können gleich oder verschieden sein und Wasserstoff, (C_1-C_6)-Alkyl, (C_3-C_7)-Cycloalkyl, (C_1-C_6)-Alkanoyl, (C_1-C_6)-Alkoxy, Halogen und Benzyloxy bedeuten.
Weiterhin können R_3 und R_4 die Nitrogruppe, die Aminogruppe, die (C_1-C_4)-mono- oder dialkylsubstituierte Aminogruppe, und die (C_1-C_6)-Alkoxy-carbonylamino-Funktion oder (C_1-C_6)-Alkoxycarbonylamino-(C_1-C_6)-alkyl-Funktion bedeuten.
Z steht für O und S.

2. N-substituierte Indol-3-gloxylamide gemäß Anspruch 1 mit der Formel 1a zur Verwendung als Antitumormittel,



Formel 1 a

wobei die Reste
R = Wasserstoff
R_1 = 4-Pyridyl, 4-Fluorophenyl
R_2 = Benzyl, 4-Chlorbenzyl, 4-Fluorbenzyl, 3-Pyridylmethyl, 4-Brombenzyl
R_3 und R_4 = Wasserstoff und
Z Sauerstoff bedeuten.

3. Pharmazeutische Zusammensetzung mit Antitumorwirkung gekennzeichnet, durch einen Gehalt an mindestens einer der Verbindungen der allgemeinen Formel 1 bzw. 1a ggf. auch sie als Säureadditionssalze, beispielsweise als Salze von Mineralsäuren, wie Salzsäure, Schwefelsäure, Phosphorsäure, Salze von organischen Säuren, wie beispielsweise Essigsäure, Milchsäure, Malonsäure, Maleinsäure, Fumarsäure, Gluconsäure, Glucuronsäure, Zitronensäure, Embonsäure, Methansulfonsäure, Trifluoressigsäure, Bernsteinsäure und 2-Hydropoxyethansulfonsäure sowie möglich, deren N-Oxide.

4. Verwendung von N-substituierten Indol-3-glyoxylamiden der allgemeinen Formel 1 bzw. 1a sowie deren physiologisch verträglichen Säureadditionssalzen zur Herstellung von Antitumormitteln zur Behandlung von Tumorerkrankungen unter Verwendung dieser Mittel und zwar insbesondere der folgenden Verbindungen bzw. deren Salze mit physiologisch verträglichen Säuren bzw. sofern möglich deren N-Oxiden:
D 24241 N-(Pyridin-4-yl)-[1-(4-fluorbenzyl)-indol-3-yl]glyoxylamid
D 24843 N-(Pyridin-4-yl)-(1-benzylindol-3-yl)-glyoxylamid
D 24850 N-(4-Fluorphenyl)-[1-(3-pyridylmethyl)-indol-3-yl]-glyoxylamid
D 24851 N-(Pyridin-4-yl)-[1-(4-chlorbenzyl)-indol-3-yl]-glyoxylamid
D-25505 N-(Pyridin-4-yl)-[1-(4-fluorbenzyl)-indol-3-yl]glyoxylamid HCL.

5. Antitumormittel enthaltend als aktiven Wirkstoff ein oder mehrere N-substituierte Indol-3-gloxylamide gemäß der allgemeinen Formel 1 bzw. 1a sowie ggf. deren physiologisch verträglichen Säureadditionssalze, insbesondere jedoch eine oder mehrere Verbindungen gemäß Anspruch 4.

6. Antitumormittel enthaltend als aktiven Wirkstoff D 24241 N-(Pyridin-4-yl)-[1-(4-fluorbenzyl)-indol-3-yl]glyoxylamid bzw. dessen Hydrochlorid.
7. Antitumormittel enthaltend als aktiven Wirkstoff D 24843 N-(Pyridin-4-yl)-(1-benzylindol-3-yl)-glyoxylamid.
8. Antitumormittel enthaltend als aktiven Wirkstoff D 24850 N-(4-Fluorphenyl)-[1-(3-pyridylmethyl)-indol-3-yl]-glyoxylamid.
9. Antitumormittel enthaltend als aktiven Wirkstoff D 24851 N-(Pyridin-4-yl)-[1-(4-chlorbenzyl)-indol-3-yl]-glyoxylamid.
10. Antitumormittel enthaltend als aktiven Wirkstoff ein oder mehrere N-substituierte Indol-3-gloxylamide gemäß der allgemeinen Formel 1 bzw. 1a sowie ggf. deren physiologisch verträglichen Säureadditionssalze und, sofern möglich, N-Oxide, insbesondere jedoch eine oder mehrere Verbindungen gemäß Anspruch 4 sowie 6 bis 8 und einen pharmazeutisch verwendbaren Träger- und/oder Verdünnungs- bzw. Hilfsstoff in Form von Tabletten, Dragees, Kapseln, Lösungen zur Infusion oder Ampullen, Suppositorien, Pflaster, inhalativ einsetzbaren Pulverzubereitungen, Suspensionen, Cremes und Salben.

Hierzu 14 Seite(n) Zeichnungen

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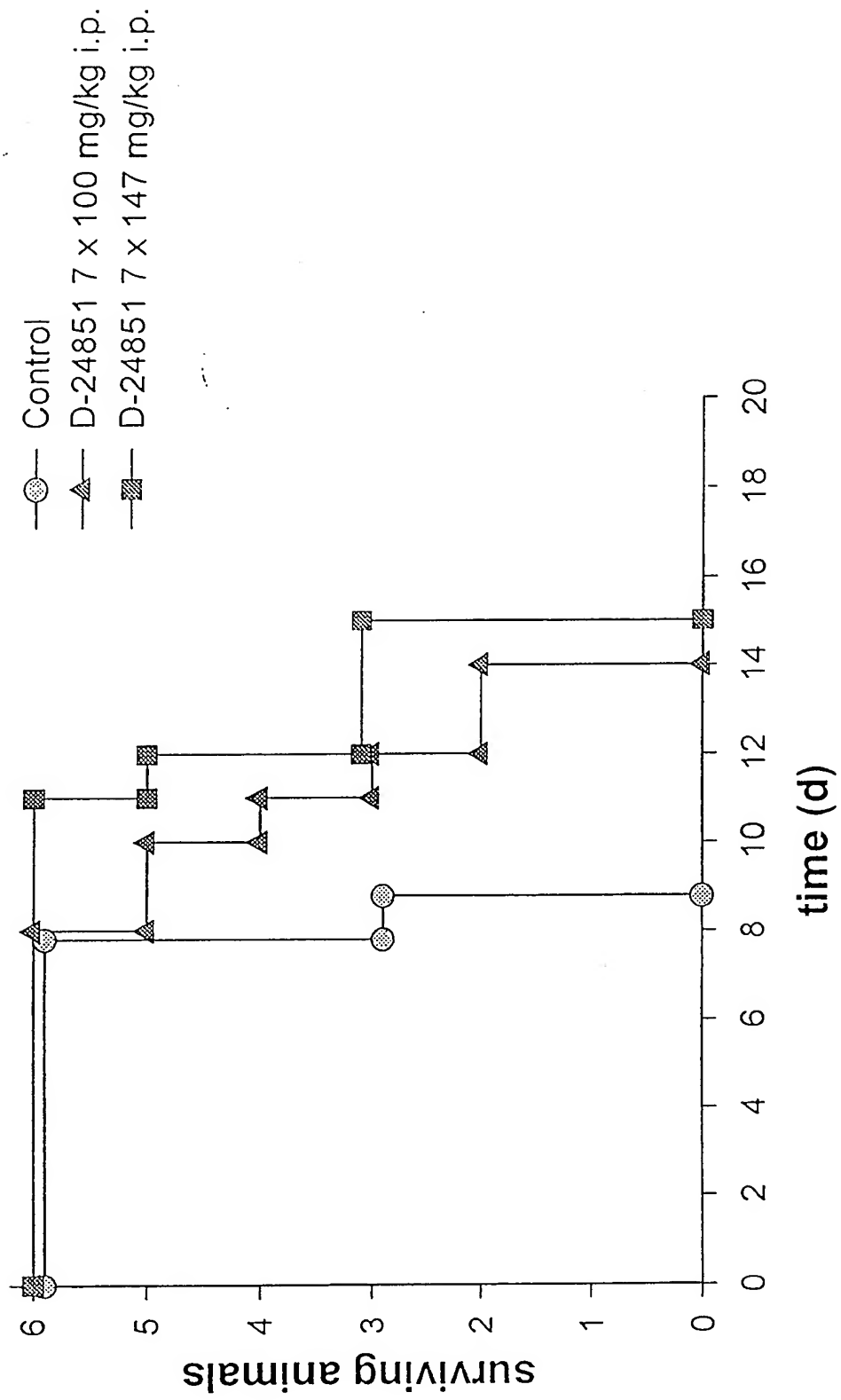
Figur 1a
Murine leucemia L 1210: D 24851 i.p.



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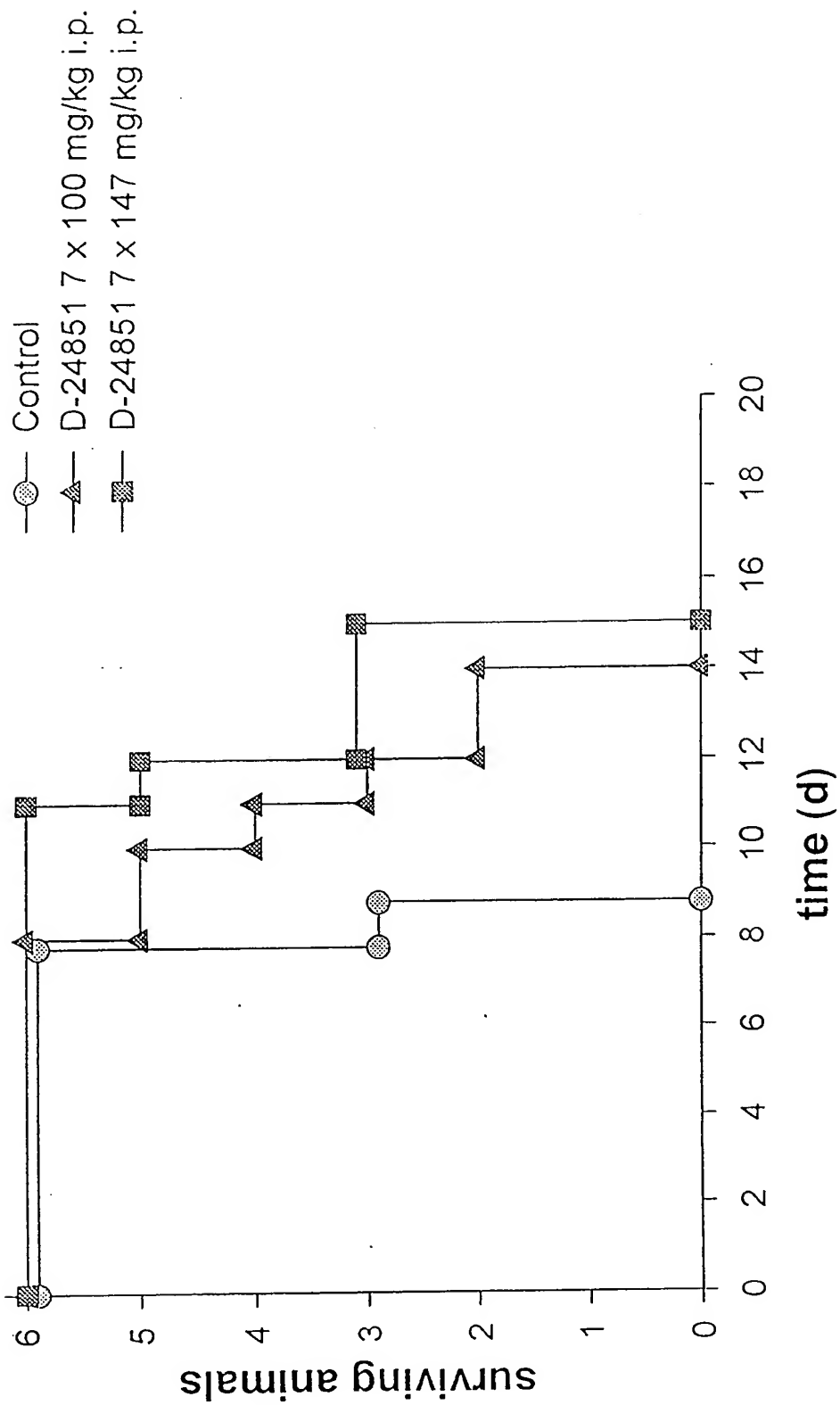
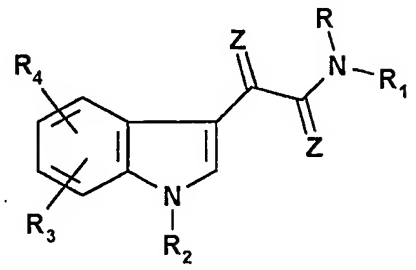
Figur 1b
Murine leucemia L 1210: D-24851 p.o.



A



Formel 1

Tabelle 1a : Indolylglyoxylamide gemäß Reaktionsschema 1

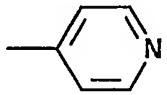
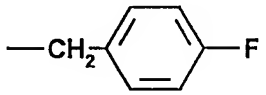
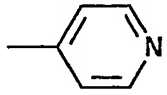
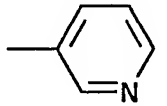
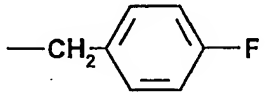
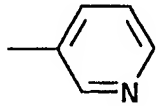
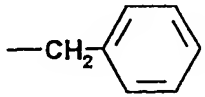
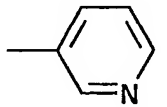
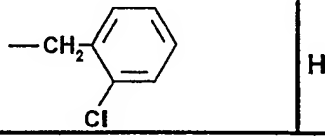
BeispielD	R	R_1	R_2	R_3	R_4	Z	Fp.
1 D-24241	H			H	H	O	225-6°C
2 D-24242	H		CH_3	H	H	O	176°C
3 D-24834	H			H	H	O	173°C
4 D-24835	H			H	H	O	140°C
5 D-24836	H			H	H	O	185°C

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Tabelle 1b: Indolylglyoxylamide gemäß Reaktionsschema 1

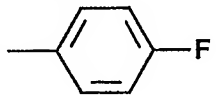
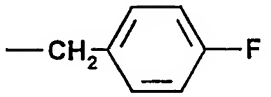
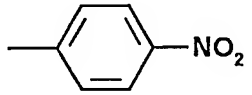
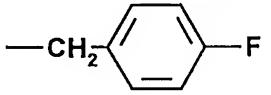
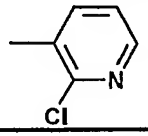
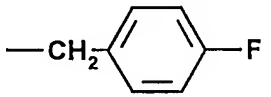
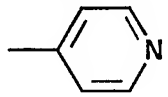
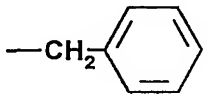
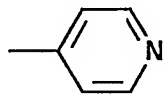
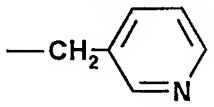
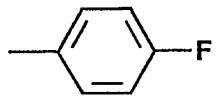
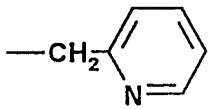
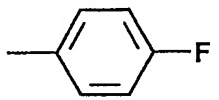
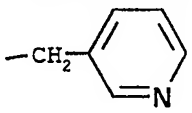
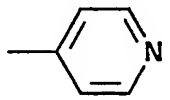
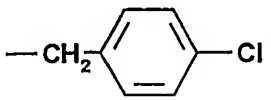
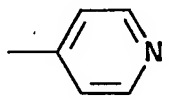
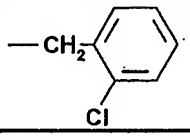
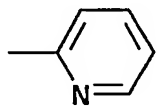
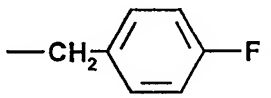
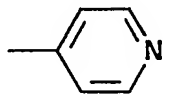
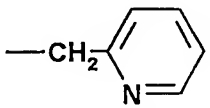
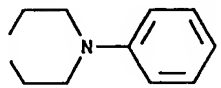
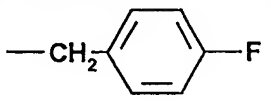
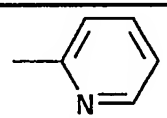
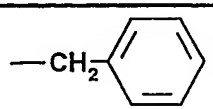
Beispiel	R	R_1	R_2	R_3	R_4	Z	Fp.
6 D-24840	H			H	H	O	199°C
7 D-24841	H			H	H	O	>250°C
8 D-24842	H			H	H	O	149°C
9 D-24843	H			H	H	O	178-180°C
10 D-24848	H			H	H	O	179°C
11 D-24849	H			H	H	O	132°C

Tabelle 1c : Indolylglyoxylamide gemäß Reaktionsschema 1

Beispiel D	R	R_1	R_2	R_3	R_4	Z	Fp.
12 D-24850	H			H	H	O	144°C
13 D-24851	H			H	H	O	262°C
14 D-24852	H			H	H	O	184°C
15 D-24853	H			H	H	O	141°C
16 D-24847	H			H	H	O	202°C
17 D-24858	R+R_1 zusam.			H	H	O	115°C
18 D-24854	H			H	H	O	112-3°C

Int. Cl.[6]:
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Tabelle 1d : Indolylglyoxylamide gemäß Reaktionsschema 1

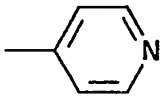
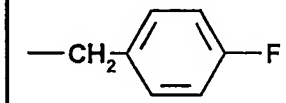
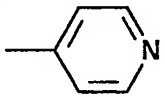
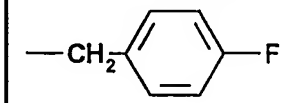
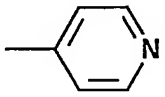
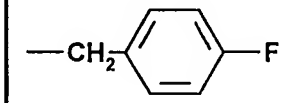
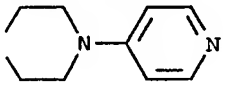
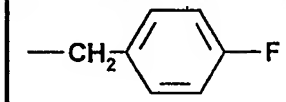
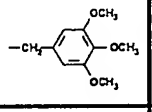
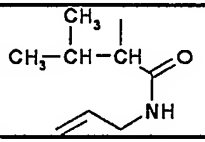
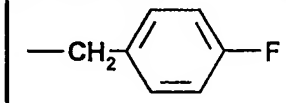
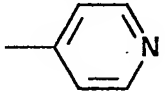
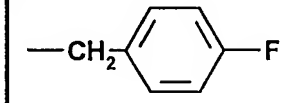
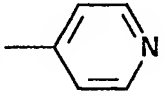
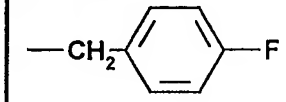
Beispiel D	R	R_1	R_2	R_3	R_4	Z	Fp.
19 D 25421	H			6-NHCOOEt	H	O	>250°C
20 D 25422	H			5-NHCOOEt	H	O	183°C
21D 25423	H			6-NHCOO- 	H	O	
22 D 25420	R+R_1 zusam. 			H	H	O	160-62°C
23 D-24866				H	H	O	139-141°C
24 D-25561	H			5-OCH_3	H	O	188°C
25 D-25559	H			5-CH_2-NHCOOEt	H	O	175-176°C

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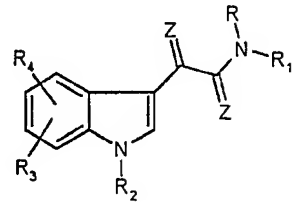
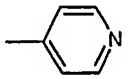
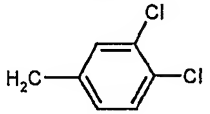
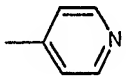
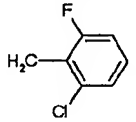
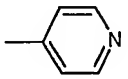
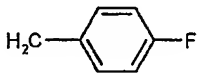
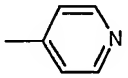
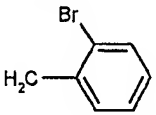
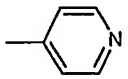
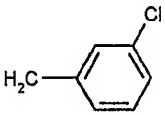
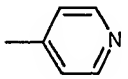
Tabelle 1e Indol-3-glyoxylsäure-Derivat gemäß Reaktionsschema 1

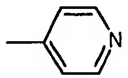
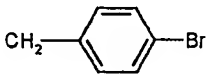
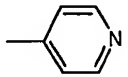
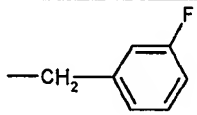
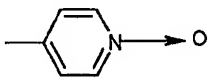
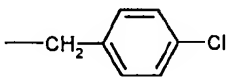
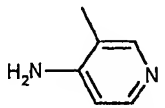
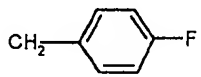
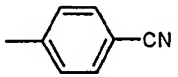
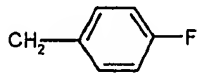
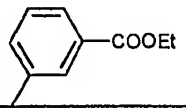
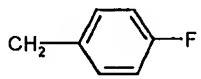
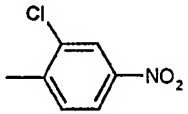
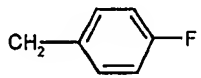
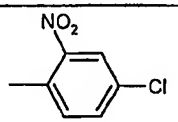
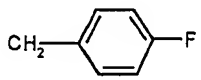


Formel 1

Beispiel, D-	R	R_1	R_2	R_3	R_4	Z	Fp.
26 D-50570	H			H	H	O	
27 D-51076	H			H	H	O	
28 D-49404	H			5-F	H	O	205-207°C
29 D-44073	H			H	H	O	192-194°C
30 D-44072	H			H	H	O	196-198°C
31 D-44067	H			H	H	O	219-221°C

Int. Cl.[6]: A 61 K 31/44
Offenlegungstag: 14. Oktober 1999

Tabelle 1f Indol-3-glyoxylsäure-Derivate gemäß Reaktionsschema 1

Beispiel, D-	R	R_1	R_2	R_3	R_4	Z	Fp.
32 D-44061	H			H	H	O	238-240°C
33 D-43163	H			H	H	O	203-205°C
34 D-51273	H			H	H	O	305-307°C
35 D-44070	H			H	H	O	>250°C
36 D-49405	H			H	H	O	237-239°C
37 D-44071	H			H	H	O	154-156°C
38 D-44069	H			H	H	O	213-215°C
39 D-44068	H			H	H	O	183-185°C

Int. Cl.[6]:
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Tabelle 1g Indol-3-glyoxylsäure-Derivate gemäß Reaktionsschema 1

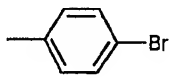
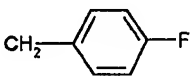
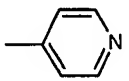
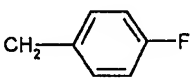
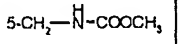
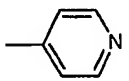
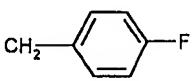
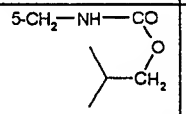
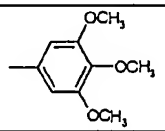
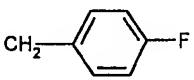
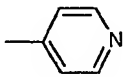
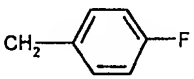
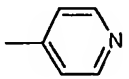
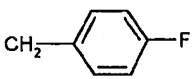
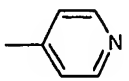
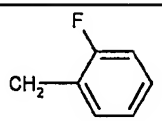
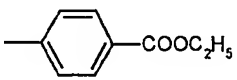
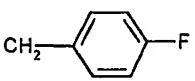
Beispiel, D-	R	R_1	R_2	R_3	R_4	Z	Fp.
40 D-44066	H			H	H	O	187-189°C
41 D-49406	H				H	O	191-193°C
42 D-49403	H				H	O	193-195°C
43 D-44064	H			H	H	O	104-106°C
44 D-43156	H			$6\text{-}NO_2$	H	O	238-240°C
45 D-43155	H			$5\text{-}NO_2$	H	O	203-205°C
46 D-43152	H			H	H	O	196-198°C
47 D-43151	H			H	H	O	141-143°C

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Tabelle 1h Indol-3-glyoxylsäure-Derivate gemäß Reaktionsschema 1

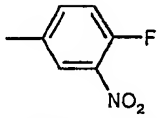
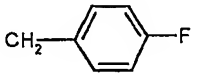
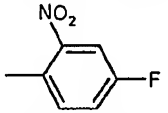
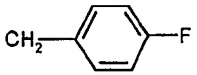
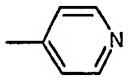
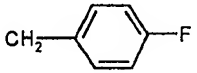
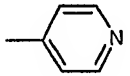
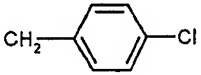
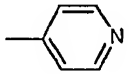
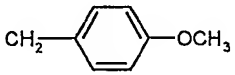
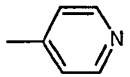
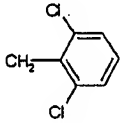
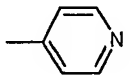
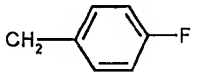
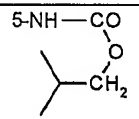
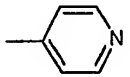
Beispiel, D-	R	R_1	R_2	R_3	R_4	Z	Fp.
48 D-43149	H			H	H	O	202-204°C
49 D-43148	H			H	H	O	183-185°C
50 D-25505 (Hydrochlorid)	H			H	H	O	Hydrochlorid
51 D-51133 (Trifluoracetat)	H			H	H	O	251-253°C Trifluoracetat
52 D-51128	H			H	H	O	173-174°C
53 D-51077	H			H	H	O	244-245°C
54 D-51195	H				H	O	228-230°C
55 D-51391	H			H	H	O	270-271°C

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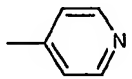
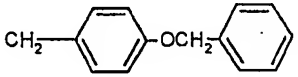
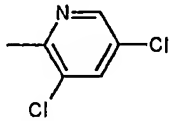
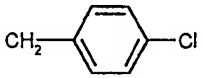
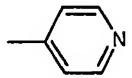
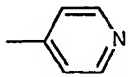
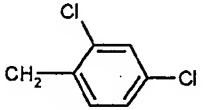
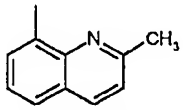
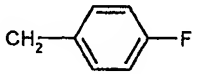
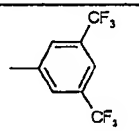
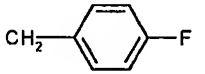
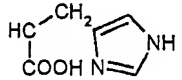
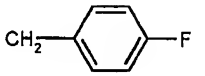
Tabelle 1 i Indol-3-glyoxylsäure-Derivate gemäß Reaktionsschema 1

Beispiel, D-	R	R_1	R_2	R_3	R_4	Z	Fp.
56 D-51393	H			H	H	O	Öl
57 D-51394	H			H	H	O	216-218°C
58 D-51184	H			H	H	O	215-217°C
59 D-51185	H			H	H	O	241-242°C
60 D-25463	H			H	H	O	°C
61 D-24584	H			H	H	O	°C
62 D-25320	H			H	H	O	145-147°C

Int. Cl.[6]:
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Tabelle 1 j Indol-3-glyoxylsäure-Derivate gemäß Reaktionsschema 1

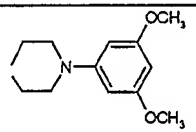
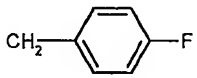
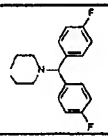
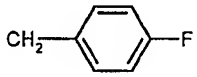
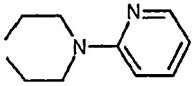
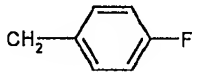
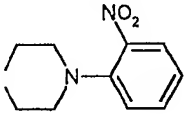
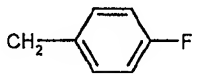
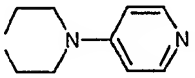
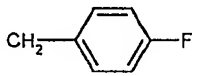
Beispiel, D-	R	R_1	R_2	R_3	R_4	Z	Fp.
63 D-51396	R_1R_1 zusammen:			H	H	O	137°C
64 D-44065	R_1R_1 zusammen:			H	H	O	205-207°C
65 D-43146	R_1R_1 zusammen:			H	H	O	89-91°C
66 D-43145	R_1R_1 zusammen:			H	H	O	68-70°C
67 D-25558	R_1R_1 zusammen:			6-$NHCOOC_2H_5$	H	O	Öl

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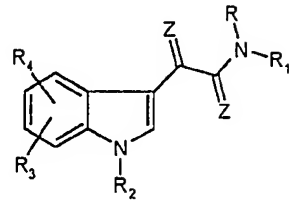
Tabelle 2 : Indolylglyoxylamide gemäß Reaktionsschema 2



Formel 1

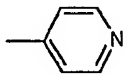
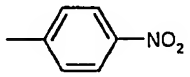
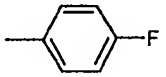
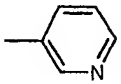
Beispiel, D-	R	R_1	R_2	R_3	R_4	Z	Fp.
1 D-24825	H		H	H	H	O	>250°C
2 D-24831	H		H	H	H	O	>250°C
3 D-24832	H		H	H	H	O	233-5°C
4 D-24833	H		H	H	H	O	235°C

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Tabelle 2a Indolylglyoxylamide gemäß Reaktionsschema 2

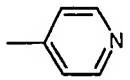
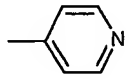
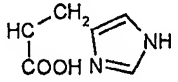
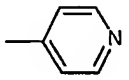
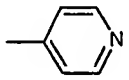
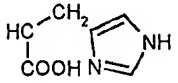
Beispiel, D-	R	R_1	R_2	R_3	R_4	Z	Fp.
5 D-43154	H		H	6-NO_2	H	O	250°C(Zers.)
6 D-43153	H		H	5-NO_2	H	O	>250°C
7 D-25319	H		H	H	H	O	156-157°C

Tabelle 2b Indol-3-glyoxylsäure-Derivate gemäß Reaktionsschema 1

Beispiel, D-	R	R_1	R_2	R_3	R_4	Z	Fp.
5 D-43154	H		H	6-NO_2	H	O	250°C(Zers.)
6 D-43153	H		H	5-NO_2	H	O	>250°C
7 D-25319	H		H	H	H	O	156-157°C

Int. Cl.[6]:
Offenlegungstag:
A 61 K 31/44
14. Oktober 1999

Exhibit D

66902 U.S. PTO
08/925326
09/08/97

PATENT APPLICATION SERIAL NO.

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

08/925326

Abstract

The invention relates to novel N-substituted indole-3-glyoxylamides, to processes for their preparation and to their pharmaceutical use. The compounds have antiasthmatic, antiallergic and immunosuppressant/immunomodulating actions.

N-substituted indol-3-glyoxylamides having anti-asthmatic, antiallergic and immunosuppressant/immuno-modulating action

~~Description~~

Background Information

Indole-3-glyoxylamides have various uses as pharmacodynamically active compounds and as synthesis components in the pharmaceutical chemistry.

The Patent Application NL 6502481 describes compounds which have an antiinflammatory and antipyretic profile of action and analgesic activity.

The British Patent GB 1 028 812 mentions derivatives of indolyl-3-glyoxylic acid and its amides as compounds having analgesic, anticonvulsant and β-adrenergic activity.

G. Domschke et al. (Ber. <u>94</u>, 2353 (1961)) describe 3-indolylglyoxylamides which are not characterized pharmacologically.

E. Walton et al. in J. Med. Chem. 11,1252 (1968) report on indolyl-3-glyoxylic acid derivatives which have an inhibitory activity on glycerophosphate dehydrogenase and lactate dehydrogenase.

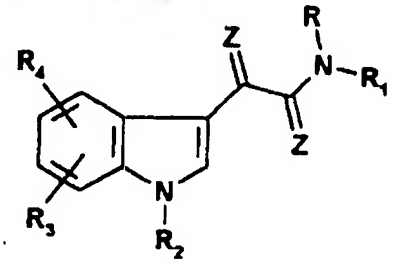
Euoropean Patent Specification EP 0 675 110 A1 describes 1H-indole-3-glyoxylamides which are profiled as sPLA2 inhibitors and are used in the treatment of septic shock, in pancreatitis, and in the treatment of allergic rhinitis and rheumatoid arthritis.

Summary of the INVENTION

The aim of the present invention is to make available novel compounds from the indolyl-3-glyoxylic acid series, which have antiasthmatic and immunomodulating action.

version of the novel compounds into medicaments and their preparation forms are furthermore described.

The subject matter of the invention comprises compounds of the general formula I,

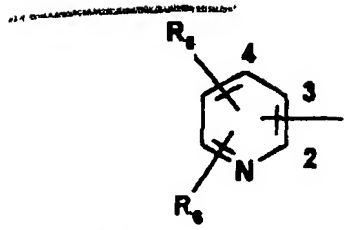


Formula I

where the radicals R, R_1, R_2, R_3, R_4 and Z have the following meaning:

R = hydrogen, (C_1-C_6)-alkyl, where the alkyl group can be mono- or polysubstituted by the phenyl ring. This phenyl ring, for its part, can be mono- or polysubstituted by halogen, (C_1-C_6)-alkyl, (C_3-C_7)-cycloalkyl, by carboxyl groups, carboxyl groups esterified with (C_1-C_6)-alkanols, trifluoromethyl groups, hydroxyl groups, methoxy groups, ethoxy groups, benzyloxy groups and by a benzyl group which is mono- or polysubstituted in the phenyl moiety by (C_1-C_6)-alkyl groups halogen atoms or trifluoromethyl groups.

R_1 can be a phenyl ring which is mono- or polysubstituted by (C_1-C_6)-alkyl, (C_1-C_6)-alkoxy, hydroxyl, benzyloxy, nitro, amino, (C_1-C_6)-alkylamino, (C_1-C_6)-alkoxy-carbonylamino and by a carboxyl group or a carboxyl group esterified by (C_1-C_6)-alkanols, or is a pyridin structure of the formula II



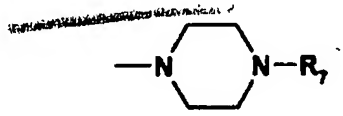
Formula II

where the pyridin structure is alternatively bonded to the ring carbon atoms 2, 3 and 4 and can be substituted by the substitutents R_5 and R_6. The radicals R_5 and R_6 can be identical or different and have the meaning (C_1-C_6)-alkyl, and also the meaning (C_3-C_7)-cycloalkyl, (C_1-C_6)-alkoxy, nitro, amino, hydroxyl, halogen and trifluoromethyl and are furthermore the ethoxy-carbonylamino radical and the group carboxy-alkyloxy in which the alkyl group can have 1-4 C atoms.

R_1 can furthermore be a 2- or 4-pyrimidinyl-heterocycle or a pyridylmethyl radical in which CH_2 can be in the 2-, 3-, 4-position where the 2-pyrimidinyl ring can be mono- or polysubstituted by the methyl group, furthermore are [sic] the 2-, 3- and 4-quinolyl structure substituted by (C_1-C_6)-alkyl, halogen, the nitro group, the amino group and the (C_1-C_6)-alkylamino radical, or are [sic] a 2-, 3- and 4-quinolylmethyl group, where the ring carbons of the pyridylmethyl and quinolylmethyl radical can be substituted by (C_1-C_6)-alkyl, (C_1-C_6)-alkoxy, nitro, amino and (C_1-C_6)-alkoxy-carbonylamino.

R_1 for the case where R is hydrogen or the benzyl group, can furthermore be the acid radical of a natural or unnatural amino acid, e.g. the α-glycyl, the α-sarcosyl, the α-alanyl, the α-

α-arginyl, the α-lysyl, the α-asparagyl and the α-glutamyl radical, where the amino groups of the respective amino acids can be present in unprotected or protected form. Possible protective groups for the amino function are the carbobenzoxy radical (Z radical) and the tert-butoxycarbonyl radical (BOC radical) and also the acetyl group. In the case of the asparagyl and glutamyl radical claimed for R_1, the second, nonbonded carboxyl group is present as a free carboxyl group or in the form of an ester with C_1-C_6-alkanols, e.g. as the methyl, ethyl or as the tert-butyl ester. R_1 can furthermore be the allylaminocarbonyl-2-methylprop-1-yl group. R and R_1, together with the nitrogen atom to which they are bonded, can furthermore form a piperazine ring of the formula III or a homopiperazine ring if R_1 is an aminoalkylene group in which



Formula III

R_7 is an alkyl radical, a phenyl ring which can be mono- or polysubstituted by (C_1-C_6)-alkyl, (C_1-C_6)-alkoxy, halogen, the nitro group, the amino function, by (C_1-C_6)-alkylamino, the benzhydryl group and the bis-p-fluorobenzylhydryl group.

R_2 can be hydrogen or the (C_1-C_6)-alkyl group, where the alkyl group can be mono- or polysubstituted by halogen and phenyl which for its part can be mono- or polysubstituted by halogen, (C_1-C_6)-alkyl, (C_3-C_7)-cycloalkyl, carboxyl groups, carboxyl groups esterified with (C_1-C_6)-alkanols, trifluoromethyl groups, hydroxyl groups, methoxy groups, ethoxy groups or benzyloxy groups. The (C_1-C_6)-alkyl group

structure, which in each case can both be mono- or polysubstituted by halogen, (C_1-C_4)-alkyl groups or (C_1-C_4)-alkoxy groups. R_2 is furthermore the aroyl radical, where the aroyl moiety on which this radical is based is the phenyl ring which can be mono- or polysubstituted by halogen (C_1-C_6)-alkyl, (C_3-C_7)-cycloalkyl, carboxyl groups, carboxyl groups esterified by (C_1-C_6)-alkanols, trifluoromethyl groups, hydroxyl groups, methoxy groups, ethoxy groups or benzyloxy groups.

R_3 and R_4 can be identical or different and are hydrogen, hydroxyl, (C_1-C_6)-alkyl, (C_3-C_7)-cycloalkyl, (C_1-C_6)-alkanoyl, (C_1-C_6)-alkoxy, halogen and benzyloxy. R_3 and R_4 can furthermore be the nitro group, the amino group, the (C_1-C_4)-mono- or dialkyl-substituted amino group, and the (C_1-C_3)-alkoxycarbonylamino function or (C_1-C_3)-alkcoxy-carbonylamino- (C_1-C_3)-alkyl function.

Z is O or S

The designation alkyl, alkanol, alkoxy or alkylamino group for the radicals R, R_1, R_2, R_3, R_4, R_5, R_6 and R_7 is normally to be understood as meaning "straight-chain" and "branched" alkyl groups, where "straight-chain alkyl groups" can be, for example, radicals such as methyl, ethyl, n-propyl, n-butyl, n-pentyl and n-hexyl and "branched alkyl groups" designate, for example, radicals such as isopropyl or tert-butyl. "Cycloalkyl" is to be understood as meaning radicals such as, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl.

The designation "halogen" represents fluorine, chlorine, bromine or iodine. The designation "alkoxy group" represents radicals such as, for example,

The compounds according to the invention can also be present as acid addition salts, for example as salts of mineral acids, such as, for example, hydrochloric acid, sulfuric acid, phosphoric acid, salts of organic acids, such as, for example, acetic acid, lactic acid, malonic acid, maleic acid, fumaric acid, gluconic acid, glucuronic acid, citric acid, embonic acid, methanesulfonic acid, trifluoroacetic acid and succinic acid.

Both the compounds of the formula I and their salts are biologically active. The compounds of the formula 1 can be administered in free form or as salts with a physiologically tolerable acid.
Administration can be carried out orally, parenterally, intravenously, transdermally or by inhalation.

The invention furthermore relates to pharmaceutical preparations containing at least one compound of the formula I or its salt with physiologically tolerable inorganic or organic acids and, if appropriate, pharmaceutically utilizable excipients and/or diluents or auxiliaries.

Suitable administration forms are, for example, tablets, coated tablets, capsules, solutions or ampoules, suppositories, patches, powder preparations which can be inhaled, suspensions, creams and ointments.

Detailed Description of the Invention

The compounds according to the invention have a good antiasthmatic, antiallergic and immunosuppressant/immunomodulating action, for example in transplantations and diseases such as psoriasis, rheumatoid disorders and chronic polyarthritis, in the following pharmacological models:

Inhibition of the "late phase" eosinophilia in the BAL 24 hours after allergen challenge in guinea pigs

Male guinea pigs (200 - 250 g, Dunkin Hartley Shoe) were actively sensitized subcutaneously with ovalbumin (10 µg of ovalbumin + 1 mg of $Al(OH)_3$) and boosted 2 weeks later. One week after boosting with ovalbumin, the animals were exposed to an inhalation challenge with ovalbumin (0.5 % strength solution) for 20 - 30 seconds. 24 hours later, the animals were killed by means of an overdose of urethane, exsanguinated and a bronchoalveolar lavage (BAL) was carried out using 2 x 5 ml of 0.9 % strength physiological saline solution.

The lavage fluid was collected and centrifuged at 400 g for 10 minutes, and the pellets were suspended in 1 ml of 0.9 % strength physiological saline solution. The eosinophils were counted microscopically in a Neubauer chamber after staining by means of Becton Dickinson test kit No. 5877. This test kit contains Phloxin B as a selective stain for eosinophils. The eosinophils in the BAL was [sic] counted here for each animal and expressed as eosinophils (millions/animal). For each group the mean value and standard deviation were determined. The percentage inhibition of eosinophilia for the group treated with test substance was calculated according to the following formula:

$$(A - B) - (B - C) / (A - C) \times 100 = \% \text{ inhibition}$$

in this formula A eosinophils correspond to the untreated challenge group, B eosinophils to the treated group and C eosinophils to the unchallenged control group.

The animals were treated with a histamine H_1 antagonist (azelastine: 0.01 mg/kg p.o.) 2 hours before allergen

hours after allergen challenge. The percentage inhibition of eosinophilia in the BAL was calculated on groups of 6 - 10 animals.

Table: Inhibition of the "late phase" - eosinophilia 24 h after allergen challenge in guinea pigs

Substance	Dose [mg/kg]	Administration	n	% Inhibition
Cyclosporin A	5	i.p. + 4h	17	50.0
	10	i.p. + 4h	11	47.0
	30	p.o. + 4h	10	68.8
According to Ex. 1	5	i.p. + 4h	10	27.8
	10	i.p. + 4h	10	55.4
	30	p.o. + 4h	9	56.1

Assays for the determination of peptidylprolyl isomerase (PPIase) activity and inhibition

The PPIase activity of the cyclophilins was measured enzymatically according to Fischer et al. (1984). After isomerization of the substrate by the peptidyl prolyl isomerase, this is accessible to chymotrypsin, which cleaves the chromophore p-nitroaniline. For the determination of inhibition of the PPIase activity by substance, recombinant human Cyp B was used. The interaction of Cyp B with a potential inhibitor was carried out as follows:

A certain concentration of purified Cyp B was incubated with 1 µM substance for 15 min. The PPIase reaction was started by addition of the substrate solution to the reaction mixture which contains HEPES buffer, chymotrypsin and either test or control samples. Under these conditions, first-order kinetics were obtained with a constant $K_{observed} = K_0 + K_{enz}$, where K_0 is the spontaneous isomerization and K_{enz} is the rate of

spectrophotometer at a constant reaction temperature of 10 °C.

The observed residual activity in the presence of various substances was compared with the cyclophilins only treated with solvent. The results were given in % residual activity. Cyclosporin A (CsA) was used as the reference compound. The inhibition of the PPIase activity was additionally checked by SDS-PAGE.

Colorimetric assay (based on the MTT test) for the non-radioactive quantification of cell proliferation and survival ability

MTT is used for the quantitative determination of cell proliferation and activation, for example, in the reaction on growth factors and cytokines such as IL-2 and IL-4 and also for the quantification of the antiproliferative or toxic effects.

The assay is based on the cleavage of yellow tetrazolium salt MTT to give purple-red formazan crystals by metabolically active cells.

The cells, cultured in a 96-hole tissue culture plate, are incubated for about 4 h with yellow MTT solution. After this incubation time, purple-red formazan salt crystals are formed. These salt crystals are insoluble in aqueous solutions, but can be dissolved by addition of solubilizer and by incubation of the plates overnight.

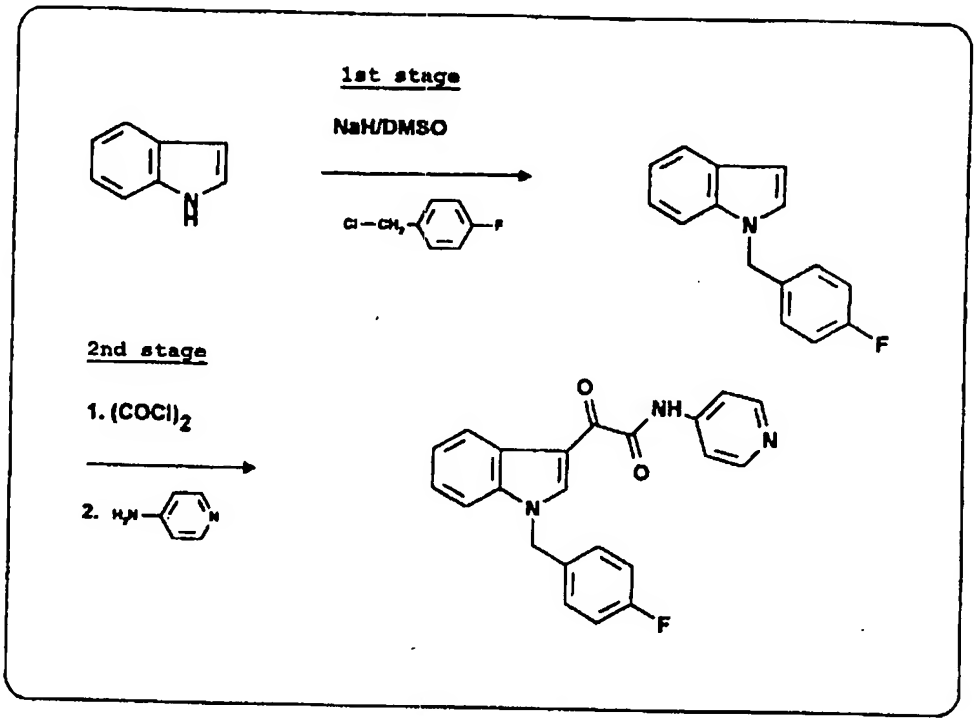
The dissolved formazan product is quantified spectrophotometrically using an ELISA reader. An increase in the number of living cells results in an increase in the total metabolic activity in the sample. This increase correlates directly with the amount of the purple-red formazan crystals formed, which are

Substance	Inhibition of PPIase activity [%]	Inhibition of CD3-induced IL-2 production [%]			Inhibition of lympho-proliferation [%]		
Conc. [µM]		0.1	1	10	0.1	1	10
According to Ex. 1	80 - 100	34	72	95	18	39	61
Cyclosporin A	80 - 100	56	82	94	8	7	11

The processes for the preparation of the compounds according to the invention are described in the following reaction schemes 1 and 2 and in general procedures. All compounds can be prepared as described or analogously.

The compounds of the general formula I are obtainable according to the following Scheme 1, shown for the synthesis of the compound Example 1:

Scheme 1



General procedure for the preparation of the compounds of the general formula I according to Scheme 1:

1st stage:

The indole derivative, which can be unsubstituted or mono- or polysubstituted on C-2 or in the phenyl structure, is dissolved in a protic, dipolar aprotic or nonpolar organic solvent, such as, for example, isopropanol, tetrahydrofuran, dimethyl sulfoxide, dimethylformamide, dimethylacetamide, N-methylpyrrolidone, dioxane, toluene or methylene chloride and added dropwise to a suspension of a base in a molar or excess amount prepared in a 3-necked flask under an N,

dimethylaminopyridine or sodium amide in a suitable solvent. The desired alkyl, aralkyl or heteroaralkyl halide, if appropriate with addition of a catalyst, such as, for example, copper, is then added and the mixture is reacted for some time, for example 30 minutes to 12 hours, and the temperature is kept within a range from 0°C to 120°C, preferably between 30°C to [sic] 80°C, particularly between 50°C and 65°C. After completion of the reaction, the reaction mixture is added to water, the solution is extracted, for example, with diethyl ether, dichloromethane, chloroform, methyl tert-butyl ether or tetrahydrofuran and the organic phase obtained in each case is dried using anhydrous sodium sulfate. The organic phase is concentrated in vacuo, the residue which remains is crystallized by trituration or the oily residue is purified by recrystallization, distillation or by column or flash chromatography on silica gel or alumina. The eluent used is, for example, a mixture of dichloromethane and diethyl ether in the ratio 8:2 (vol/vol) or a mixture of dichloromethane and ethanol in the ratio 9:1 (vol/vol).

2nd stage

The N-substituted indole obtained by the abovementioned 1st stage procedure is dissolved under a nitrogen atmosphere in an aprotic or nonpolar organic solvent, such as, for example, diethyl ether, methyl tert-butyl ether, tetrahydrofuran, dioxane, toluene, xylene, methylene chloride or chloroform and added to a solution, prepared under a nitrogen atmosphere, of a simply molar up to 60 percent excess amount of oxalyl chloride in an aprotic or nonpolar solvent, such as, for example, in diethyl ether, methyl tert-butylether, tetrahydrofuran, dioxane, toluene, xylene, methylene

preferably between 20°C and 80°C, particularly between 30°C and 50°C, for a period of 30 minutes up to 5 hours and the solvent is then evaporated. The residue of the "indolyl-3-glyoxylic acid chloride" formed in this manner which remains is dissolved in an aprotic solvent such as, for example, tetrahydrofuran, dioxane, diethyl ether, toluene or alternatively in a dipolar aprotic solvent, such as, for example, dimethylformamide, dimethylacetamide or dimethyl sulfoxide, cooled to a temperature between 10°C and -15°C, preferably between -5°C and 0°C, and treated in the presence of an acid scavenger with a solution of the primary or secondary amine in a diluent.

Possible diluents are the solvents used above for the dissolution of the indolyl-3-glyoxylic acid chloride. Acid scavengers used are triethylamine, pyridin, dimethylaminopyridine, basic ion exchanger, sodium carbonate, potassium carbonate, powdered potassium hydroxide and excess primary or secondary amine employed for the reaction. The reaction takes place at a temperature from 0°C to 120°C, preferably at 20 - 80°C, particularly between 40°C and 60°C. After a reaction time of 1 - 3 hours and standing at room temperature for 24 hours, the hydrochloride of the acid scavenger is filtered, the filtrate is concentrated in vacuo, and the residue is recrystallized from an organic solvent or purified by column chromatography on silica gel or alumina. The eluent used is, for example, a mixture of dichloromethane and ethanol (95:5, vol/vol).

Working Examples

According to this general procedure for Stages 1 and 2, on which the synthesis Scheme 1 is based, the following

compounds and their melting points can be seen from the general formula I and the substituents R_1-R_4 and Z:

Example 1

N-(Pyridin-4-yl)-[1-(4-fluorobenzyl)indol-3-yl] glyoxylamide

1st stage

1-(4-Fluorobenzyl)indole

A solution of 11.72 g (0.1 mol) of indole in 50 ml of dimethyl sulfoxide is added to a mixture of 2.64 g of sodium hydride (0.11 mol, mineral oil suspension) in 100 ml of dimethyl sulfoxide. The mixture is heated for 1.5 hours at 60°C, then allowed to cool and 15.9 g (0.11 mol) of 4-fluorobenzyl chloride are added dropwise. The solution is warmed to 60°C, allowed to stand overnight and then poured into 400 ml of water with stirring. The mixture is extracted several times with a total of 150 ml of methylene chloride, the organic phase is dried using anhydrous sodium sulfate and filtered, and the filtrate is concentrated in vacuo. The residue is distilled in a high vacuum: 21.0 g (96% of theory)
B.p. (0.5 mm): 140°C

2nd stage

N-(pyridin-4-yl)-[1-(4-fluorobenzyl)indol-3-yl] glyoxylamide

A solution of 4.75 g (21.1 mmol) of 1-(4-fluorobenzyl)indole in 25 ml of ether is added dropwise at 0°C and under N_2 to a solution of 2.25 ml of oxalyl chloride in 25 ml of ether. The mixture is refluxed for

and the solution is cooled to -5°C and treated dropwise with a solution of 4.66 g (49.5 mmol) of 4-aminopyridine in 200 ml of THF. The mixture is refluxed for 3 hours and allowed to stand at room temperature overnight. The 4-aminopyridine hydrochloride is filtered off with suction, the precipitate is washed with THF, the filtrate is concentrated in vacuo and the residue is recrystallized from ethyl acetate.

Yield: 7.09 g (90% of theory)

Melting point: 225-226°C

Elemental analysis:

Calc.	C	70.77	H	4.32	N	11.25
Found	C	71.09	H	4.36	N	11.26

Example 2	N-(Pyridin-4-yl)-(1-methylindol-3-yl) glyoxylamide
Example 3	N-(Pyridin-3-yl)-[1-(4-fluorobenzyl)-indol-3-yl]glyoxylamide
Example 4	N-(Pyridin-3-yl)-(1-benzylindol-3-yl) glyoxylamide
Example 5	N-(Pyridin-3-yl)-[1-(2-chlorobenzyl)-indol-3-yl]glyoxylamide
Example 6	N-(4-Fluorophenyl)-[1-(4-fluorobenzyl)-indol-3-yl]glyoxylamide
Example 7	N-(4-Nitrophenyl)-[1-(4-fluorobenzyl)-indol-3-yl]glyoxylamide
Example 8	N-(2-Chloropyridin-3-yl)-[1-(4-fluoro-benzyl)indol-3-yl]glyoxylamide
Example 9	N-(Pyridin-4-yl)-(1-benzylindol-3-yl)-glyoxylamide
Example 10	N-(Pyridin-4-yl)-[1-(3-pyridylmethyl)-indol-3-yl]glyoxylamide

Example 12	N-4(Fluorophenyl)-[1-(3-pyridylmethyl)-indol-3-yl]glyoxylamide
Example 13	N-(Pyridin-4-yl)-[1-(4-chlorobenzyl)-indol-3-yl]glyoxylamide
Example 14	N-(Pyridin-4-yl)-[1-(2-chlorobenzyl)-indol-3-yl]glyoxylamide
Example 15	N-(Pyridin-2-yl)-[1-4-fluorobenzyl)-indol-3-yl]glyoxylamide
Example 16	N-(Pyridin-4-yl)-[1-(2-pyridylmethyl)-indol-3-yl]glyoxylamide
Example 17	(4-Phenylpiperazin-1-yl)-[1-(4-fluorobenzyl)indol-3-yl]glyoxylamide
Example 18	N-(Pyridin-2-yl)-(1-benzylindol-3-yl)-glyoxylamide
Example 19	N-(Pyridin-4-yl)-[1-(4-fluorobenzyl)-6-ethoxycarbonylaminoindol-3-yl]-glyoxylamide
Example 20	N-(Pyridin-4-yl)-[1-(4-fluorobenzyl)-5-ethoxycarbonylaminoindol-3-yl]-glyoxylamide
Example 21	N-(Pyridin-4-yl)-[1-(4-fluorobenzyl)-6-cyclopentyloxycarbonylaminoindol-3-yl]-glyoxylamide
Example 22	4-(Pyridin-4-yl)-piperazin-1-yl)-[1-(4-fluorobenzyl)indol-3-yl]-glyoxylamide
Example 23	N-(3,4,5-Trimethoxybenzyl)-N-(allyl-aminocarbonyl-2-methylprop-1-yl)-[1-(4-fluorobenzyl)indol-3-yl]glyoxylamide
Example 24	N-(Pyridin-4-yl)-[1-(4-fluorobenzyl)-5-methoxyindol-3-yl]glyoxylamide
Example 25	N-(Pyridin-4-yl)-[1-(4-fluorobenzyl)-5-hydroxyindol-3-yl]glyoxylamide
Example 26	N-pyridin-4-yl-[1-(4-fluorobenzyl)-5-ethoxycarbonylaminomethylindol-3-yl]-glyoxylamide

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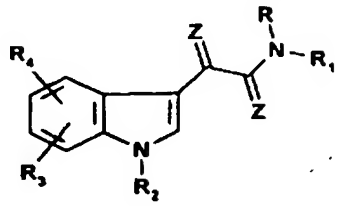
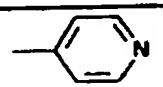
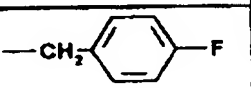
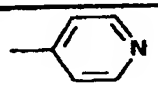
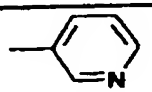
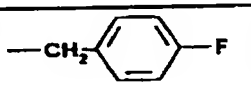
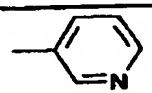
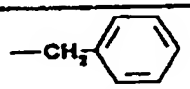
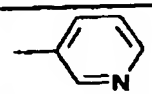
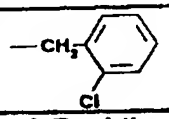
Formula 1



Example	R	R_1	R_2	R_3	R_4	Z	M.p.
Ex. 1	H			H	H	O	225-6°C
Ex. 2	H		CH_3	H	H	O	176°C
Ex. 3	H			H	H	O	173°C
Ex. 4	H			H	H	O	140°C
Ex. 5	H			H	H	O	185°C

1: Novel indolylglyoxylamides according to reaction Scheme 1

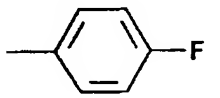
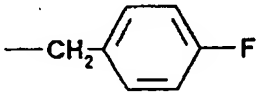
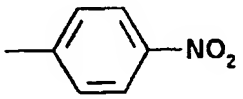
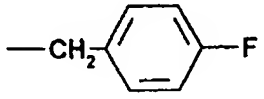
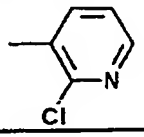
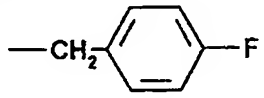
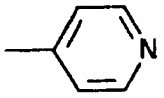
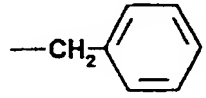
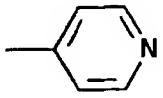
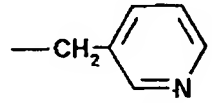
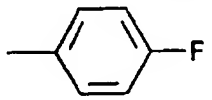
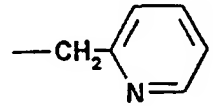
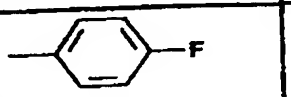
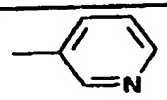
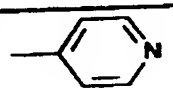
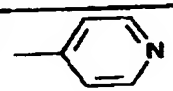
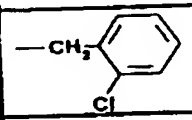
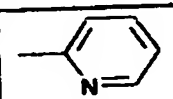
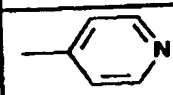
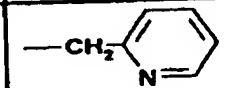
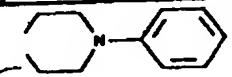
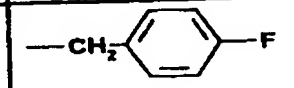
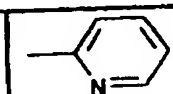
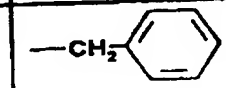
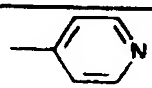
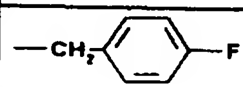
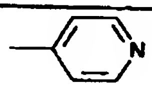
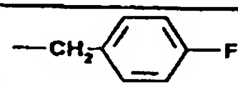
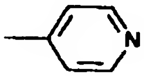
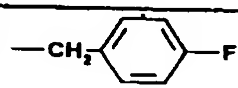
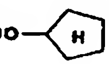
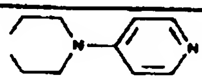
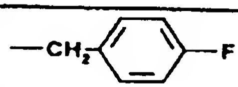
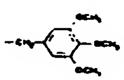
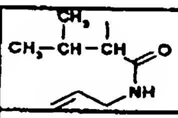
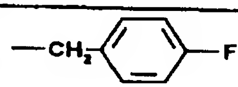
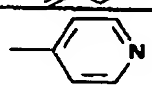
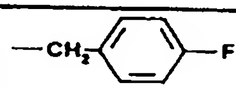
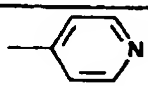
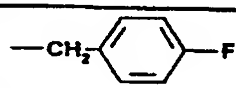
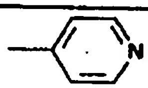
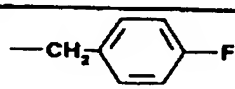
Example	R	R_1	R_2	R_3	R_4	Z	M.p.
Ex. 6	H			H	H	O	199°C
Ex. 7	H			H	H	O	>250°C
Ex. 8	H			H	H	O	149°C
Ex. 9	H			H	H	O	178-180°C
Ex. 10	H			H	H	O	179°C
Ex. 11	H			H	H	O	132°C

Table 1: Novel indolylglyoxylamides according to reaction Scheme 1

Le	R	R_1	R_2	R_3	R_4	Z	M.p.
12	H			H	H	O	144°C
13	H			H	H	O	234°C
14	H			H	H	O	184°C
15	H			H	H	O	141°C
16	H			H	H	O	202°C
17	R+R_1 ~~zusam.~~ together			H	H	O	115°C
18	H			H	H	O	112-3°C

1: Novel indolylglyoxylamides according to reaction Scheme 1

T5 21

le	R	R1	R2	R3	R4	Z	M.p.
19	H			6-NHCOOEt	H	O	>250°C
20	H			6-NHCOOEt	H	O	183°C
21	H			6-NHCOO— 	H	O	oily ~~Oil~~
22	R+R1 ~~zusam.~~ together			H	H	O	160-62°C
23				H	H	O	139-141°C
24	H			5-OCH_3	H	O	188°C
25	H			5-OH	H	O	>250°C
26	H			5-CH_2-NHCOOEt	H	O	175-176°C

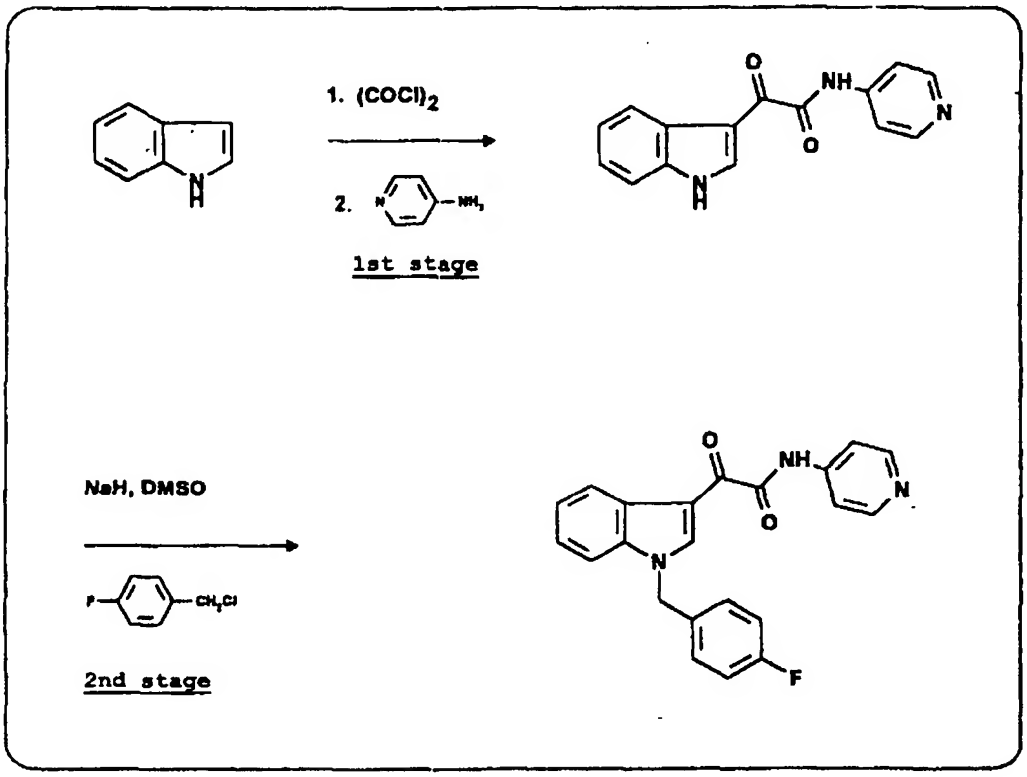
Novel indolylglyoxylamides according to reaction Scheme 1

Starting materials for the compounds of the general formula 1 prepared according to synthesis Scheme 1, which come from Table 1

All precursors for the final synthesis stages of Examples 1 to 22 and 24 to 26 are commercially available.

Furthermore, the compounds of the general formula I are also obtainable according to the synthesis route of Scheme 2, shown by the synthesis of the compound Example 27:

Scheme 2



General procedure for the preparation of the compounds of the general formula 1 according to Scheme 2

1st stage:

The indole derivative dissolved in a solvent, such as given above for oxalyl chloride, which can be unsubstituted or substituted on C-2 or in the phenyl ring, is added dropwise at a temperature between -5°C and +5°C to a solution of a simply molar up to 60% excess amount of oxalyl chloride prepared under a nitrogen atmosphere in an aprotic or nonpolar solvent, such as, for example, in diethyl ether, methyl tert-butyl ether, tetrahydrofuran, dioxane or alternatively dichloromethane. The reaction solution is then heated for 1 to 5 hours to a temperature between 10°C and 120°C, preferably between 20°C and 80°C, particularly between 30°C and 60°C, and the solvent is then evaporated. The residue of the (indol-3-yl)glyoxylic acid chloride which remains is dissolved or suspended in an aprotic solvent, such as, for example, tetrahydrofuran, dioxane, diethyl ether, toluene or alternatively in a dipolar aprotic solvent, such as, for example, dimethylformamide, dimethylacetamide or dimethyl sulfoxide, cooled to a temperature between -10°C and +10°C, preferably to -5°C to 0°C, and treated with a solution of the primary or secondary amine in a diluent in the presence of an acid scavenger. Possible diluents are the solvents used for the dissolution of the "indolyl-3-glyoxylic acid chloride". Acid scavengers used are triethylamine, pyridin, dimethylaminopyridine, basic ion exchanger, sodium carbonate, potassium carbonate, powdered potassium hydroxide and excess primary or secondary amine employed for the reaction. The reaction takes place at a temperature from 0°C to 120°C, preferably at

with water, and the solid is filtered off with suction and dried in vacuo. The desired compound is purified by recrystallization in an organic solvent or by column chromatography on silica gel or alumina. The solvent used is, for example, a mixture of dichloromethane and ethanol (10:1, vol/vol).

2nd stage

The "indol-3-ylglyoxylamide" obtained according to the abovementioned 1st Stage procedure is dissolved in a protic, dipolar aprotic or nonpolar organic solvent, such as, for example, in isopropanol, tetrahydrofuran, dimethyl sulfoxide, dimethylformamide, dimethylacetamide, N-methylpyrrolidone, dioxane, toluene or methylene chloride and added dropwise to a suspension of a base such as, for example, sodium hydride, powdered potassium hydroxide, potassium tert-butoxide, dimethylaminopyridine or sodium amide in a suitable solvent, in a molar amount or in excess prepared in a 3-necked flask under an N_2 atmosphere. The desired alkyl, aralkyl or heteroaralkyl halide is then added either in undiluted form or in a diluent which was also used, for example, to dissolve the "indol-3-yl glyoxylamide", if appropriate with addition of a catalyst, such as, for example, copper, and the mixture is allowed to react for some time, e.g. 30 minutes to 12 hours, and the temperature is kept within a range between 0°C and 120°C, preferably between 30°C and 80°C, particularly between 50 and 70°C. After completion of the reaction, the reaction mixture is added to water, the solution is extracted, for example, with diethyl ether, dichloromethane, chloroform, methyl tert-butyl ether, tetrahydrofuran or N-butanol and the organic phase obtained in each case is dried using anhydrous sodium sulfate.

The organic phase is concentrated in vacuo, the residue

chromatography or flash chromatography on silica gel or alumina. The eluent used is, for example, a mixture of methylene chloride and diethyl ether in the ratio 8:2 (vol/vol) or a mixture of methylene chloride and ethanol in the ratio 9:1 (v/v).

Working Examples

According to this general procedure for Stages 1 and 2, on which synthesis Scheme 2 is based, compounds were synthesized which have already been prepared according to the synthesis course of reaction Scheme 1 and are evident from Table 1. The relevant precursors of these compounds are evident from Table 2.

Example 27

N-(pyridin-4-yl)-[1-(4-flurobenzyl)indol-3-yl]-glyoxylamide

(Final substance, identical to Example 1)

1st stage

N-(Pyridin-4-yl)-(indol-3-yl)glyoxylamide

A solution of 10 g (85.3 mmol) of indole in 100 ml of ether is added dropwise at 0°C to a solution of 9 ml of oxalyl chloride in 100 ml of anhydrous ether. The mixture is kept under reflux for 3 hours. A suspension of 12 g (127.9 mmol) of 4-aminopyridine in 500 ml of tetrahydrofuran is then added dropwise at -5°C, and the reaction mixture is heated to reflux temperature with stirring for 3 hours and allowed to stand overnight at room temperature. The precipitate is filtered and treated with water and the dried compound is purified on a silica gel column (silica gel 60, Merck AG,

Yield: 9.8 g (43.3% of theory)

M.p.: from 250°C

2nd stage

N-(Pyridin-4-yl)-[1-[4-fluorobenzylindol-3-yl]glyoxylamide

The N-(pyridin-4-yl)-(indol-3-yl)glyoxylamide obtained according to the 1st stage is reacted with 4-fluorobenzyl chloride according to the "benzylation procedure" (Page 11) and the compound obtained is isolated.

Yield: 41% of theory

M.p.: 224-225°C

Elemental analysis:

Calc.	C 70.77	H 4.32	N 11.25
Found	C 70.98	H 4.40	N 11.49

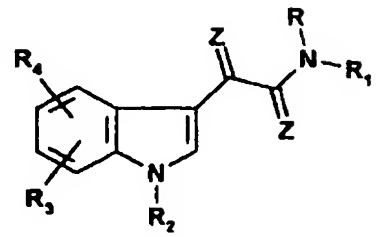
Example 28 N-(4-Nitrophenyl)-[1-(4-fluorobenzyl)-indol-3-yl]glyoxylamide
(Final substance, identical to Example 7)

Example 29 N-(4-Fluorophenyl)-[1-(4-fluorobenzyl)-indol-3-yl]glyoxylamide
(Final substance, identical to Example 6)

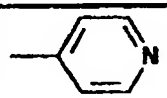
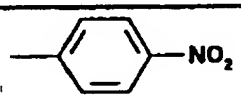
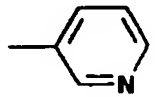
Example 30 N-)Pyridin-3-yl)-[1-(4-fluorobenzyl)-indol-3-yl]glyoxylamide
(Final substance, identical to Example 3)

The following precursors (1st stage of reaction scheme 2, Table 2) were obtained according to the present Scheme 2.

Example 31	N-(Pyridin-4-yl)-(indol-3-yl)-glyoxylamide
Example 32	N-(4-Nitrophenyl)-(indol-3-yl)-glyoxylamide
Example 33	N-(4-Fluorophenyl)-(indol-3-yl)-glyoxlyamide
Example 34	N-(Pyridin-3-yl)-(indol-3-yl)-glyoxylamide



Formula 1

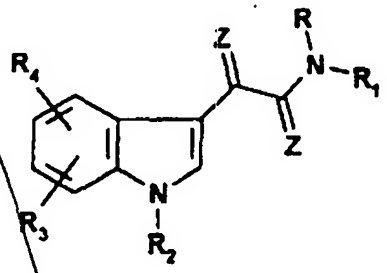
ample	R	R_1	R_2	R_3	R_4	Z	M.p.
. 31	H		H	H	H	O	>250°C
. 32	H		H	H	H	O	>250°C
. 33	H		H	H	H	O	233-6°C
. 34	H		H	H	H	O	235°C

: Novel indolylglyoxylamides according to reaction Scheme 2

~~Patent Claims~~

WHAT IS CLAIMED IS

1. **N-substituted indol-3-glyoxylamides of the formula 1**

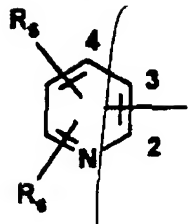


I

and their acid addition salts,
where the radicals R, R_1, R_2, R_3, R_4 and Z have the following meaning:

R = hydrogen, (C_1-C_6)-alkyl, where the alkyl group can be mono- or polysubstituted by the phenyl ring. This phenyl ring, for its part, can be mono- or polysubstituted by halogen, (C_1-C_6)-alkyl, (C_3-C_7)-cycloalkyl, by carboxyl groups, carboxyl groups esterified with (C_1-C_6)-alkanols, trifluoromethyl groups, hydroxyl groups, methoxy groups, ethoxy groups, benzyloxy groups and by a benyl [sic] group which is mono- or polysubstituted in the phenyl moiety by (C_1-C_6)-alkyl groups halogen atoms or trifluoromethyl groups,

R_1 can be a phenyl ring which is mono- or polysubstituted by (C_1-C_6)-alkyl, (C_1-C_6)-alkoxy, hydroxyl, benzyloxy, nitro, amino, (C_1-C_6)-alkylamino, (C_1-C_6)-alkoxy-carbonylamino and by a carboxyl group or a carboxyl group esterified by (C_1-C_6)-alkanols, or is a pyridin structure of the formula II



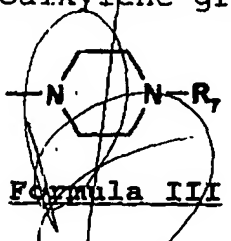
Formula II

where the pyridin structure is alternatively bonded to the ring carbon atoms 2, 3 and 4 and can be substituted by the substitutents R_5 and R_6. The radicals R_5 and R_6 can be identical or different and have the meaning (C_1-C_6)-alkyl, and also the meaning (C_3-C_7)-cycloalkyl, (C_1-C_6)-alkoxy, nitro, amino, hydroxyl, halogen and trifluoromethyl and are furthermore the ethoxy-carbonylamino radical and the group carboxy-alkyloxy in which the alkyl group can have 1-4 C atoms,

R_1 can furthermore be a 2- or 4-pyrimidinyl-heterocycle or a pyridylmethyl radical in which CH_2 can be in the 2-, 3-, 4-position where the 2-pyrimidinyl ring can be mono- or polysubstituted by the methyl group, furthermore are [sic] the 2-, 3- and 4-quinolyl structure substituted by (C_1-C_6)-alkyl, halogen, the nitro group, the amino group and the (C_1-C_6)-alkylamino radical, or are [sic] a 2-, 3- and 4-quinolyl methyl group, where the ring carbons of the pyridylmethyl and quinolylmethyl radical can be substituted by (C_1-C_6)-alkyl, (C_1-C_6)-alkoxy, nitro, amino and (C_1-C_6)-alkoxy-carbonylamino,

R_1 for the case where R is hydrogen or the benzyl group, can furthermore be the acid radical of a natural or unnatural amino acid, e.g. the α-

α-arginyl, the α-lysyl, the α-asparagyl and the α-glutamyl radical, where the amino groups of the respective amino acids can be present in unprotected or protected form and are possible protective groups for the amino function of the carbobenzoxy radical (Z radical) and the tert-butoxycarbonyl radical (BOC radical) and also the acetyl group. In the case of the asparagyl and glutamyl radical claimed for R_1, the second, nonbonded carboxyl group is present as a free carboxyl group or in the form of an ester with C_1-C_6-alkanols, e.g. as the methyl, ethyl or as the tert-butyl ester. R_1 can furthermore be the allylaminocarbonyl-2-methylprop-1-yl group. R and R_1, together with the nitrogen atom to which they are bonded, can furthermore form a piperazine ring of the formula III or a homopiperazine ring if R_1 is an aminoalkylene group in which



Formula III

R_7 is an alkyl radical, a phenyl ring which can be mono- or polysubstituted by (C_1-C_6)-alkyl, (C_1-C_6)-alkoxy, halogen, the nitro group, the amino function, by (C_1-C_6)-alkylamino, the benzhydryl group and the bis-p-fluorobenzylhydryl group,

R_2 can be hydrogen or the (C_1-C_6)-alkyl group, where the alkyl group can be mono- or polysubstituted by halogen and phenyl which for its part can be mono- or polysubstituted by halogen, (C_1-C_6)-alkyl, (C_3-C_7)-cycloalkyl, carboxyl groups, carboxyl groups esterified with (C_1-C_6)-alkanols, trifluoromethyl groups, hydroxyl groups, methoxy groups, ethoxy

structure, which in each case can both be mono- or polysubstituted by halogen, (C_1-C_4)-alkyl groups or (C_1-C_4)-alkoxy groups. R_2 is furthermore the aroyl radical, where the aryl' moiety on which this radical is based is the phenyl ring which can be mono- or polysubstituted by halogen, (C_1-C_6)-alkyl, (C_3-C_7)-cycloalkyl, carboxyl groups, carboxyl groups esterified by (C_1-C_6)-alkanols, trifluoromethyl groups, hydroxyl groups, methoxy groups, ethoxy groups or benzyloxy groups,

R_3 and R_4 can be identical or different and are hydrogen, hydroxyl, (C_1-C_6)-alkyl, (C_3-C_7)-cycloalkyl, (C_1-C_6)-alkanoyl, (C_1-C_6)-alkoxy, halogen and benzyloxy. R_3 and R_4 can furthermore be the nitro group, the amino group, the (C_1-C_4)-mono- or dialkyl-substituted amino group, and the (C_1-C_3)-alkoxycarbonylamino function or the (C_1-C_3)-alkoxycarbonylamino- (C_1-C_3)-alkyl function,

Z is O or S,

and where the designation alkyl, alkanol, alkoxy or alkylamino group for the radicals R, R_1, R_2, R_3, R_4, R_5, R_6 and R_7 is normally to be understood as meaning "straight-chain" and "branched" alkyl groups, where "straight-chain alkyl groups" can be, for example, radicals such as methyl, ethyl, n-propyl, n-butyl, n-pentyl and n-hexyl and "branched alkyl groups" designate, for example, radicals such as isopropyl or tert-butyl. "Cycloalkyl" is to be understood as meaning radicals such as, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl, additionally the designation "halogen" represents fluorine, chlorine, bromine or iodine, and the designation "alkoxy group" represents radicals such as, for example, methoxy, ethoxy, propoxy, butoxy,

B3 3. A compound ~~Compounds~~ according to Claim 1 selected from the group consisting of

N-(Pyridin-4-yl)-[1-(4-fluorobenzyl)indol-3-yl]-glyoxylamide

N-(Pyridin-4-yl)-(4-methylindol-3-yl)glyoxylamide

N-(Pyridin-3-yl)-[1-(4-fluorobenzyl)-indol-3-yl]-glyoxylamide

N-(Pyridin-3-yl)-(1-benzylindol-3-yl)glyoxylamide

N-(Pyridin-3-yl)-[1-(2-chorobenzyl)indol-3-yl]-glyoxylamide

N-(4-Fluorophenyl)-[1-(4-fluorobenzyl)indol-3-yl]-glyoxylamide

N-(4-Nitrophenyl)-[1-(4-fluorobenzyl)indol-3-yl]-gloxylamide

N-(2-Chloropyridine-3-yl)-[1-(4-fluorobenzyl)indol-3-yl]glyoxylamide

N-(Pyridin-4-yl)-(-benzylindol-3-yl)glyoxylamide

N-(Pyridin-4-yl)-[1-(3-pyridylmethyl)indol-3-yl]-glyoxylamide

N-(4-Fluorophenyl)-[1-(2-pyridylmethyl)indol-3-yl]-glyoxyamide

N-(4-Fluorophenyl)-[1-(3-pyridylmethyl)indol-3-yl]-glyoxylamide

N-(Pyridin-4-yl)-[1-(4-chlorobenzyl)indol-3-yl]-

N-(Pyridin-4-yl)-[1-(2-chlorobenzyl)indol-3-yl]-glyoxylamide

N-(Pyridin-2-yl)-[1-(4-fluorobenzyl)indol-3-yl]-glyoxylamide

N-(Pyridin-4-yl)-[1-(2-pyridylmethyl)indol-3-yl]-glyoxylamide

~~(4-Phenylpiperazin-1-yl)-[1-(4-fluorobenzyl)indol-3-yl]-glyoxylamide~~

N-(Pyridin-2-yl)-(1-benzylindol-3-yl)glyoxylamide

~~4-(Pyridin-4-yl)-piperazin-1-yl)-[1-(4-fluorobenzyl)-indol-3-yl]glyoxylamide~~

N-(Pyridin-4-yl)-[1-(4-fluorobenzyl)-6-ethoxycarbonyl-aminoindol-3-yl]glyoxylamide

N-(Pyridin-4-yl)-[1-(4-fluorobenzyl)-5-ethoxycarbonyl-aminoindol-3-yl]glyoxylamide

N-(Pyridin-4-)-[1-(4-fluorobenzyl)-6-cyclopentyl-oxycarbonylaminoindol-3-yl]glyoxylamide

N-(3,4,5-Trimethoxybenzyl)-N-(allylaminocarbonyl-2-methylprop-1-yl)-[1-(4-fluorobenzyl)indol-3-yl]-glyoxylamide

N-(Pyridin-4-yl)-[1-(4-fluorobenzyl)-5-methoxyindol-3-yl]glyoxylamide

N-(Pyridin-4-yl)-[1-(4-fluorobenzyl)-5-hydroxyindol-3-yl]glyoxylamide

N-(Pyridin-4-yl)-[1-(4-fluorobenzyl)-5-ethoxycarbonyl

3. Use of the compounds of the formula I according to one of claims 1 and 2 for the production of a medicament.

4. Use of the compounds of the formula I according to claims 1 to 3 on their own or in combination with one another for the production of a medicament having antiasthmatic, antiallergic and immunosuppressant/immunomodulating action for transplantation and diseases such as, for example, psoriasis, rheumatoid disorders and chronic polyarthritis.

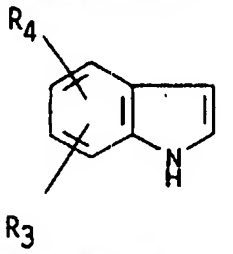
5. Medicaments comprising at least one compound of the formula I according to one of claims 1 and 2 in addition to customary excipients and/or diluents or auxiliaries.

6. Process for the production of a medicament, characterized in that a compound of the formula I according to one of claims 1 and 2 is processed with customary pharmaceutical excipients and/or diluents or other auxiliaries to give pharmaceutical preparations or brought into a therapeutically useable form.

7. Medicaments according to claims 1 to 6 in the form of tablets, coated tablets, capsules, solutions or ampoules, suppositories, patches, powder preparations which can be employed by inhalation, suspensions, creams and ointments.

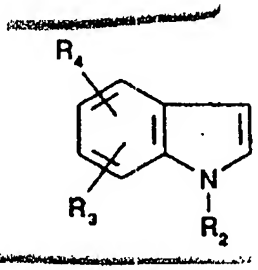
8. Process for the preparation of N-substituted indole-3-glyoxylamides of the formula I according to claims 1 and 2, in which R, R_1, R_2, R_3, R_4 and Z have the meaning mentioned in claim 1, characterized in that

a) an indole derivative of the formula IV



IV

in which R_3 and R_4 have the meaning mentioned, is added to a suspended base in a protic, dipolar aprotic or nonpolar organic solvent, reacted with a reactive compound which carries the radical R_2 and where R_2 has the meaning mentioned, the 1-indole derivative of the formula V



V

in which R_2, R_3 and R_4 have the meaning mentioned, is reacted with a reactive compound of the formula VI

$$(C\text{-}Z\text{-}Hal)_2 \qquad VI$$

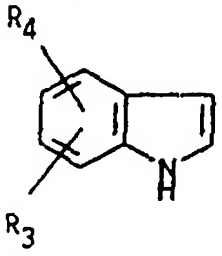
in which Z has the meaning oxygen and Hal is a halogen fluorine, chlorine, bromine or iodine, and then with a primary or secondary amine of the formula VII

$$HNRR_1 \qquad VII$$

in which R and R_1 have the meaning mentioned, in an aprotic or dipolar aprotic solvent and the target compound of the formula I is isolated,

or

b) an indole derivative of the formula IV



IV

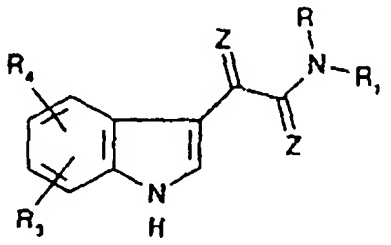
in which R_3 and R_4 have the meaning mentioned, is reacted in an aprotic or nonpolar solvent with a reactive compound of the formula VI

$$(C\text{-}Z\text{-}Hal)_2 \qquad VI$$

in which Z has the meaning oxygen and Hal is a halogen fluorine, chlorine, bromine or iodine, and then in an aprotic or dipolar aprotic solvent with a primary or secondary amine of the formula VII

$$HNRR_1 \qquad VII$$

in which R and R_1 have the meaning mentioned, and then the 3-indole derivative of the formula VIII



VIII

in which R, R_1, R_2, R_3, R_4 and Z have the meaning mentioned, is reacted in a protic, dipolar aprotic or nonpolar organic solvent in the presence of a suspended base with a reactive compound which carries the radical R_2 and where R_2 has the meaning mentioned, and the target compound of the formula I is isolated.

A/NO Fee

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FOR FILING APPLICATION
Under Rule 53(a), (b)(l) & (d)(l)
(No Filing Fee or Oath/Declaration)
(Do NOT use for Provisional or PCT Applications)
Use for Design or Utility Applications

PATENT APPLICATION

RULE 53(d) NO DECLARATION

Honorable Commissioner of Patents and Trademarks
Washington, DC 20231

Atty. Dkt. 241249 (M#) | 96/09PH (Client Ref)

Date: MONDAY, September 8, 1997

Sir:

1. This is a Request for filing a new Patent Application(☐ Design ☒ Utility) entitled:

2. (Complete) Title: N-substituted indole-3-glyoxylamides having anti-asthmatic, antiallergic and immunosuppressant/immuno-modulating action

without a filing fee or Oath/Declaration but for which is enclosed the following:

3. ☒ Abstract 1 page(s).

4. 35 Pages of Specification (only spec. and claims); 5. ☐ Specification in non-English language

6. 8 Numbered claim(s); and

7. ☐ _____ sheet(s) per set: ☐ 1 set informal; 8. ☐ formal of size: ☐ A4 ☐ 11" Drawings:

9. **DOMESTIC/INTERNATIONAL** priority is claimed under 35 USC 119(e)/120/365(c) based on the following provisional, nonprovisional and/or PCT international application(s):

Application No.	Filing Date	Application No.	Filing Date
(1)		(2)	
(3)		(4)	
(5)		(6)	

10. **FOREIGN** priority is claimed under 35 USC 119(a)-(d)/365(b) based on filing in GERMANY

Application No.	Filing Date	Application No.	Filing Date
(1) 19636150.8	06 SEP 1996	(2)	
(3)		(4)	
(5)		(6)	

11. 1 (No.) Certified copy (copies): ☒ attached; ☐ previously filed (date) _____ in U.S. Application No. / filed on _____

12. ☐ This is a reissue of Patent No. _____

13. ☐ **Amend the specification** by inserting before the first line - This is a ☐ Continuation-in-Part ☐ Divisional ☐ Continuation ☐ Substitute Application (MPEP 201.09) of:

13(a) ☐ National Appln. No. / filed .- - (M#)

13(b) ☐ International Appln. No. PCT/ filed _____ which designated the U.S. - -

14. ☐ See top of first page re continuing appln (X box only if info is there)

14(a) extension to date: ☐ concurrently filed ☐ not needed ☐ previously filed

15. ☐: Prior application is assigned to

by Assignment recorded ____________________ Reel ________ Frame ____________

16. [X] Attached:

Translation verification certificate

17. This application is made by the following named inventor(s) **(Double check instructions for accuracy):**

(1) Inventor	Guillaume		LEBAUT	
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(5) Inventor	Stefan		SZELENYI	
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18. NOTE: FOR ADDITIONAL INVENTORS, check box ☒
and attach sheet with same information regarding additional inventors.

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REQUEST FOR FILING APPLICATION
Under Rule 53(a), (b)(l) & (d)(l)
(Continued : Additional Inventors)

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Post Office Address			
(include Zip Code)			

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Post Office Address			
(include Zip Code)			

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(include Zip Code)			

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Post Office Address			
(include Zip Code)			

(12) Inventor			
	First	Middle Initial	Family Name
Residence			
	City	State/Foreign Country	Country of Citizenship

APPLICATION UNDER UNITED STATES PATENT LAWS

Invention: N-substituted indole-3-glyoxylamides having anti-asthmatic, antiallergic and immunosuppressant/immuno-modulating action

Inventor (s): LEBAUT, Guillaume
MENCIU, Cecilia
KUTSCHER, Bernhard
EMIG, Peter
SZELENYI, Stefan
BRUNE, Kay

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This is a:

- ☐ Provisional Application
- ☒ Regular Utility Application
- ☐ Continuing Application
- ☐ PCT National Phase Application
- ☐ Design Application
- ☐ Reissue Application
- ☐ Plant Application
- ☐ Substitute Specification Filed _____

SPECIFICATION

IN THE UNITED STATES PATENT OFFICE

I, John William SPICER BSc PhD MRSC CChem,
translator to RWS Translations Ltd., of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England, declare:

1. That I am a citizen of the United Kingdom of Great Britain and Northern Ireland.

2. That I am well acquainted with the German and English languages.

3. That the attached is, to the best of my knowledge and belief, a true translation into the English language of the specification in German filed with the application for a patent in the U.S.A. on
under the number

4. That I believe that all statements made herein of my own knowledge are true and that all statements made on information and belief are true; and further that these statements are made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the patent application in the United States of America or any patent issuing thereon.

J.W. Spicer

For and on behalf of RWS Translations Ltd.